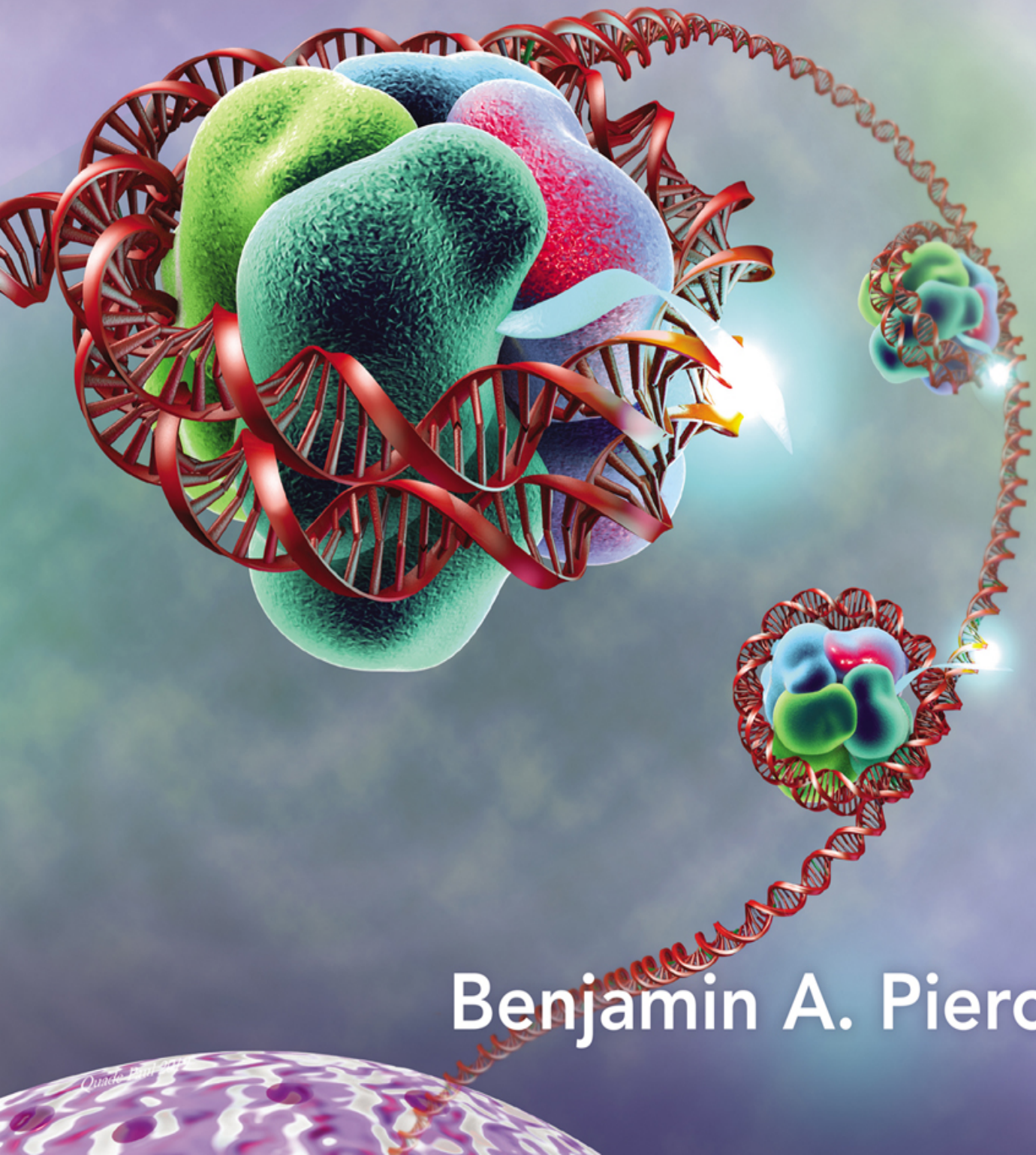
Genetics

A Conceptual Approach

Seventh Edition



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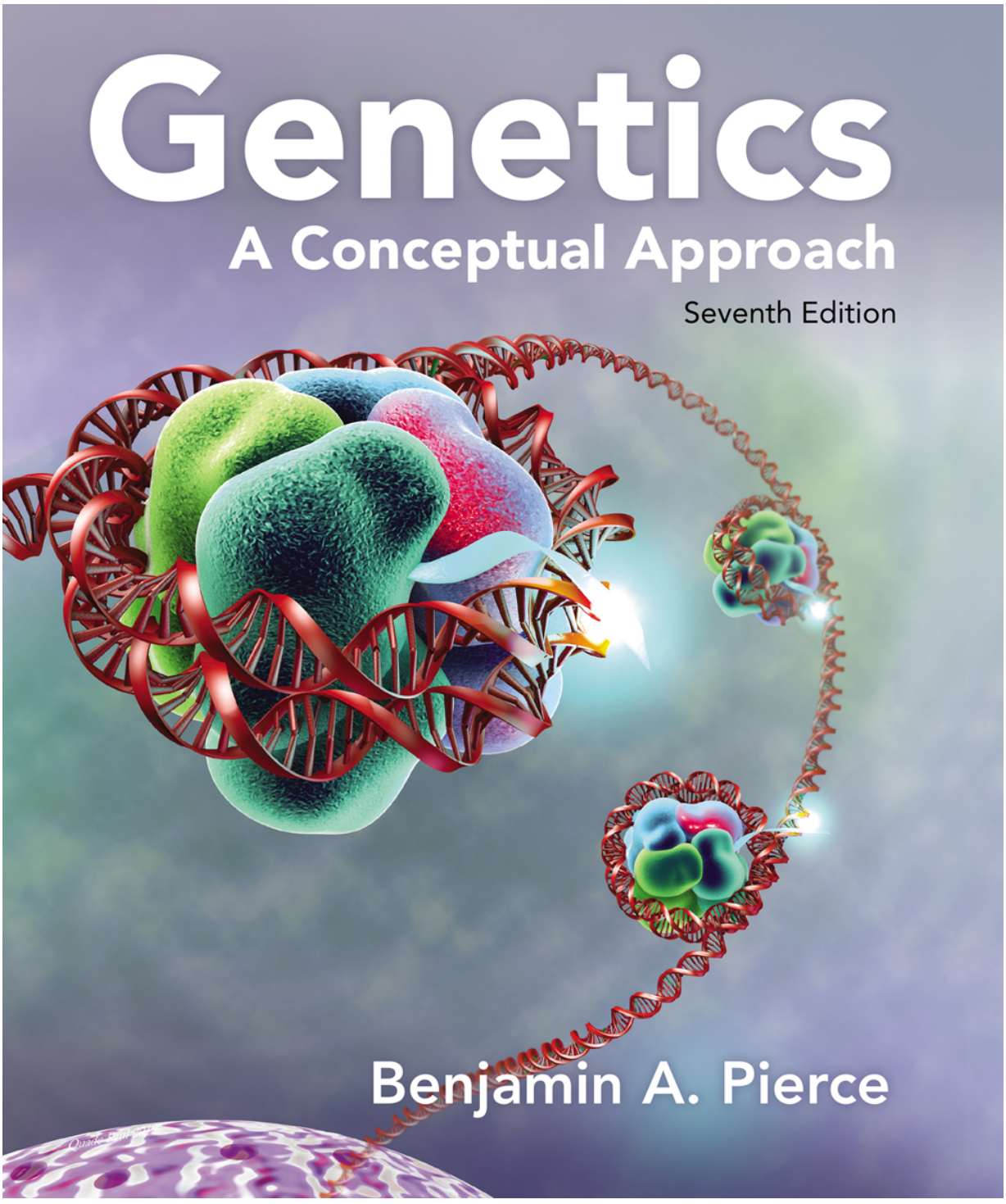

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The book cover shows a string of helical D N A wrapped around nucleosomes. Text at the bottom right reads, Benjamin A. Pierce.

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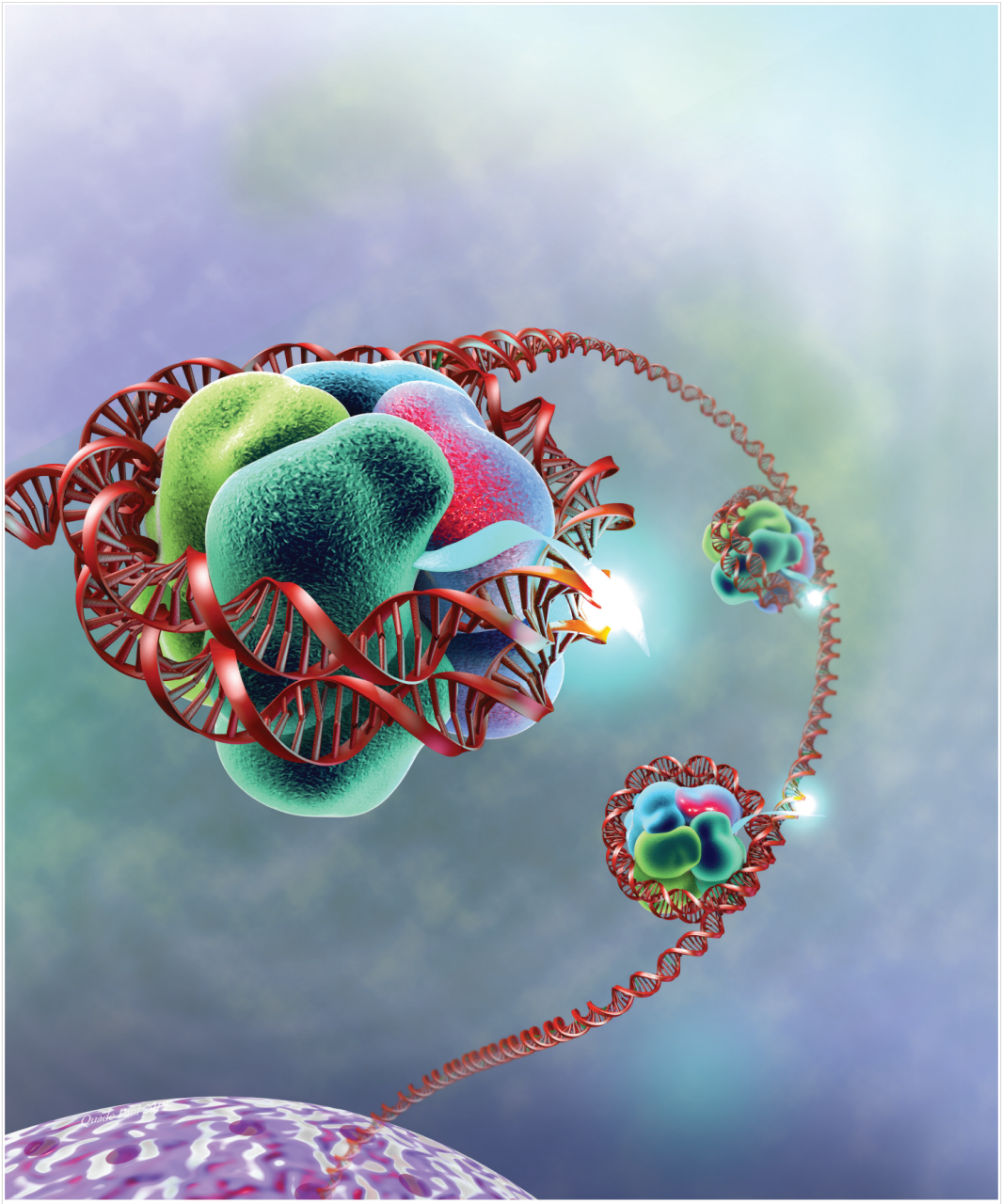
Benjamin A. Pierce
Southwestern University

Austin • Boston • New York • Plymouth



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proudly continued the legacy of providing revolutionary, quality educational tools for teaching and learning in STEM.

To my parents, Rush and Amanda Pierce; my children, Sarah Pierce Dumas and Michael Pierce; and my genetic partner, friend, and soul mate for 39 years, Marlene Tyrrell

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Letter from the Author

Marlene Tyrrell

I still remember the excitement I felt when I was in your place, taking my first genetics course. I was intrigued by the principles of heredity, which allow one to predict what offspring will look like even before they are born; I was fascinated to learn that these principles have their foundation in the chemistry of an elegant molecule called DNA; and I was captivated to find that genetics underlies evolution, the process responsible for life's endless diversity and beauty. These elements of genetics still impress and excite me today. One of the great things about teaching genetics is the chance to convey that excitement to students.

This book has been written in different places: in my office at Southwestern University, on the back porch of my home overlooking the hills of central Texas, in the mountains of northern New Mexico, and in airports and hotel rooms around the country. Regardless of location, whenever I write, I try to imagine that I'm sitting with a small group of students, having a conversation about genetics. My goal as the author of *Genetics: A Conceptual Approach* is to have that conversation with you. I want to become a trusted guide on your journey through introductory genetics. In this book, I've tried to share some of what I've learned in my years of teaching genetics. I provide advice and encouragement at places where students often have difficulty, and I tell stories of the people, places, and experiments of genetics—past and present—to keep the subject relevant, interesting, and alive. My goal is to help you learn the necessary details, concepts, and problem-solving skills while

encouraging you to see the elegance and beauty of the larger landscape.

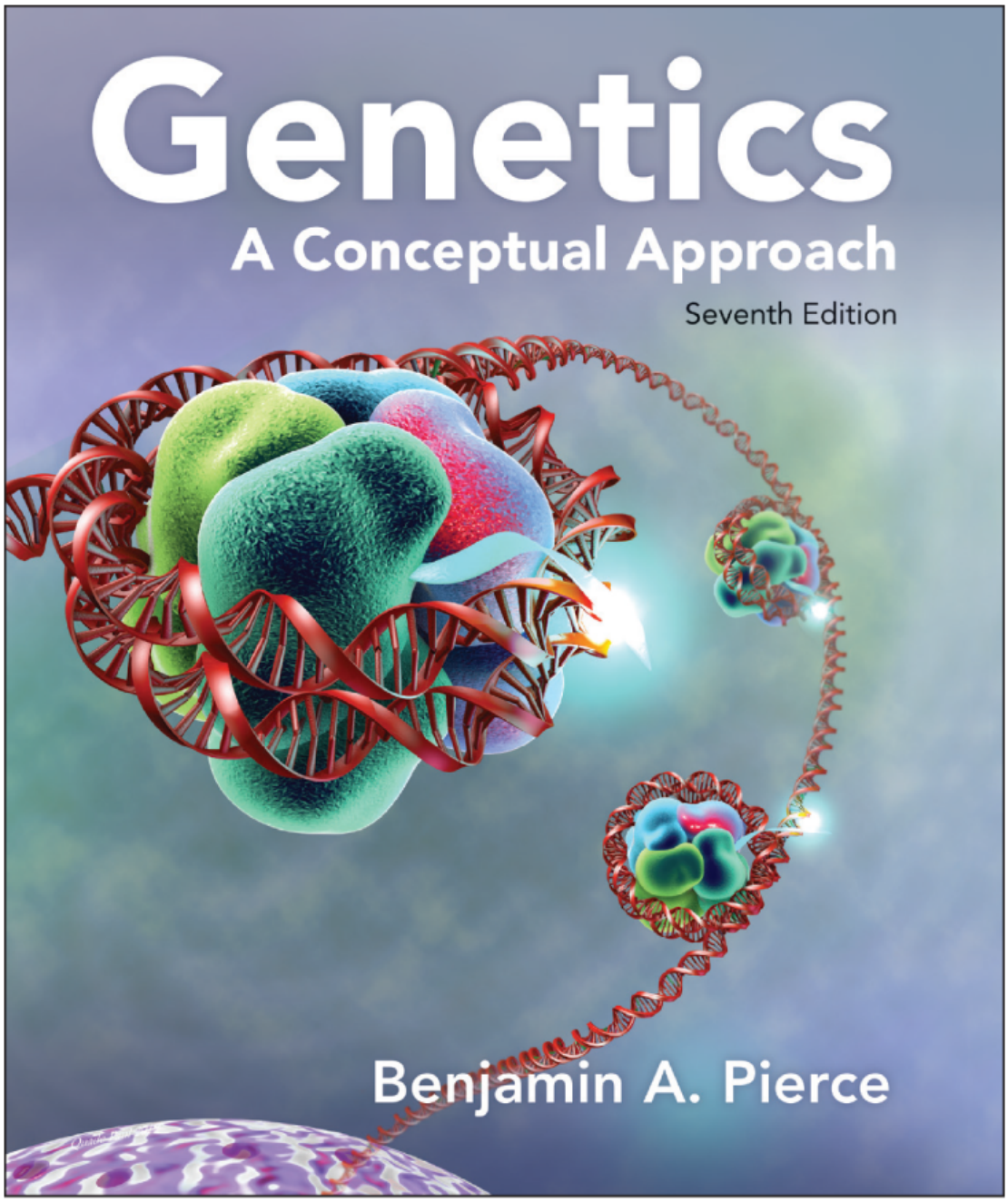
Educational research demonstrates that students learn best when they are actively engaged with their subject, and this has been my own experience in teaching and learning. In this seventh edition of *Genetics: A Conceptual Approach,* I wanted to share some active learning tools that I use in my own classroom: think-pair-share and concept mapping questions—exercises designed for small group work—as well as extensive problem sets at the end of each chapter. Additional group learning exercises and digital learning tools are provided on the SaplingPlus web site. I encourage you to take advantage of these resources and work with other students in learning genetics.

At Southwestern University, my office door is always open, and my students often drop by to share their experiences, concerns, and triumphs. I learn as much from my students as they learn from me, and I would love to learn from you—by email (pierceb@southwestern.edu), by telephone (512-863-1974), or in person (Southwestern University, Georgetown, Texas).

Ben Pierce
Professor of Biology
and Holder of the Lillian Nelson Pratt Chair
Southwestern University

A conceptual approach to success in genetics

With ***Genetics: A Conceptual Approach,*** Ben Pierce brings a master teacher's experience to his introductory genetics textbook, clarifying this complex subject by focusing on the big picture of genetics concepts and how they connect to one another.



Cover and Title Page Illustration: Echo Medical Media

Description

The book cover shows a string of helical D N A wrapped around nucleosomes. Text at the bottom left reads, Benjamin A. Pierce.

The new seventh edition continues this mission by expanding upon the powerful pedagogy and tools that have made this title so successful. New question types, more learning guidelines for students, and an updated art program round out a powerful text. Improvements to the online resources in SaplingPlus give students the conceptual and problem solving understanding they need for success.

New to the seventh edition:

CONNECTING PROBLEMS allow students to connect concepts across chapters. These questions ask students to integrate what they have learned over several chapters, and to apply what they learned to problem solving or data analysis.

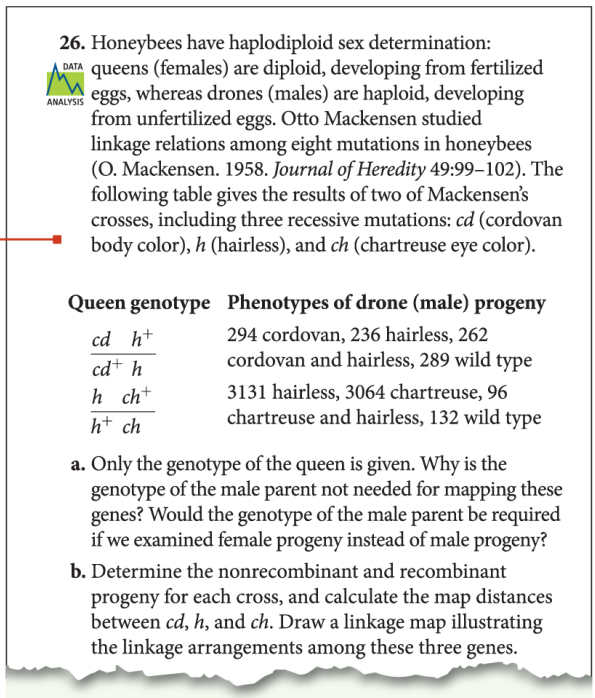
CONCEPT MAPPING EXERCISES help students conceptually map out topics within a chapter by including key terms and describing how they are connected. Creating connections between concepts and ideas is critically important for effective learning. These new concept mapping exercises are designed to help students build an understanding of the relationships among important concepts and terms by creating a visual map that depicts these relationships. These problems can be assigned as homework, or students can work on them in groups, allowing them to discuss how to organize the concepts in a map.

END-OF-CHAPTER LEARNING OBJECTIVES help frame the concepts covered in each section. A set of Learning Objectives, one for each major section of the chapter (followed by key concepts for that section), helps students identify the main learning goals for each chapter.

Powerful tools for problem solving

> ***Genetics: A Conceptual Approach*** features powerful tools, both in the text and online in SaplingPlus, to teach and reinforce the problem-solving skills students need for success in genetics.

DATA ANALYSIS PROBLEMS draw on examples from published research articles and ask students to apply the concepts they learned to analyze real data.



26. Honeybees have haplodiploid sex determination: queens (females) are diploid, developing from fertilized eggs, whereas drones (males) are haploid, developing from unfertilized eggs. Otto Mackensen studied linkage relations among eight mutations in honeybees (O. Mackensen. 1958. *Journal of Heredity* 49:99–102). The following table gives the results of two of Mackensen's crosses, including three recessive mutations: *cd* (cordovan body color), *h* (hairless), and *ch* (chartreuse eye color).

Queen genotype	Phenotypes of drone (male) progeny
$\frac{cd \quad h^+}{cd^+ \quad h}$	294 cordovan, 236 hairless, 262 cordovan and hairless, 289 wild type
$\frac{h \quad ch^+}{h^+ \quad ch}$	3131 hairless, 3064 chartreuse, 96 chartreuse and hairless, 132 wild type

a. Only the genotype of the queen is given. Why is the genotype of the male parent not needed for mapping these genes? Would the genotype of the male parent be required if we examined female progeny instead of male progeny?

b. Determine the nonrecombinant and recombinant progeny for each cross, and calculate the map distances between *cd*, *h*, and *ch*. Draw a linkage map illustrating the linkage arrangements among these three genes.

Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

Description

The sample text shows a portion of a page with the following text, Honeybees have haplodiploid sex determination: queens (females) are diploid, developing from fertilized eggs, whereas drones (males) are haploid, developing from unfertilized eggs. Otto Mackensen studied linkage relations among eight mutations in honeybees (O. Mackensen. 1958. Journal of Heredity 49: 99 to 102). The following table gives the results of two of Mackensen's crosses, including three recessive mutations: small C small D (cordovan body color), small H (hairless), and small C small H (chartreuse eye color). A table with two rows and two columns is present below. The column headers are as follows: Queen genotype and Phenotypes of drone (male) progeny. The row entries are as follows:

Row 1: small C small D small H superscript positive on upper chromosome and small C small D superscript positive small H on bottom chromosome; 294 cordovan, 236 hairless, 262 cordovan and hairless, 289 wild type.

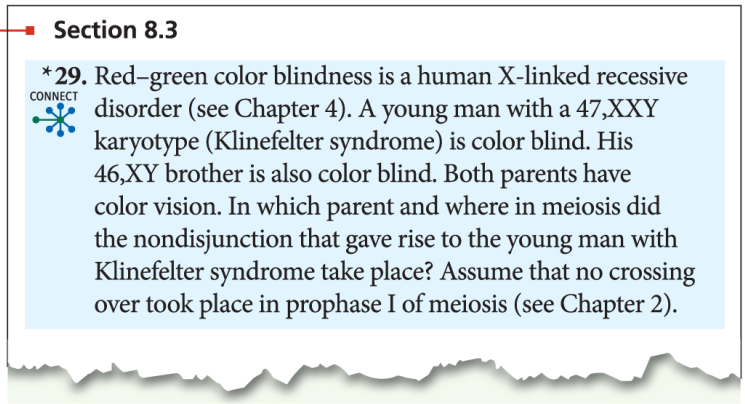
Row 2: small H small C small H superscript positive on upper chromosome and small H superscript positive small C small H on bottom chromosome; 3131 hairless, 3064 chartreuse, 96 chartreuse and hairless, 132 wild type.

Text below reads, a. Only the genotype of the queen is given. Why is the genotype of the male parent not needed for mapping these genes? Would the genotype of the male parent

be required if we examined female progeny instead of male progeny?

b. Determine the nonrecombinant and recombinant progeny for each cross, and calculate the map distances between small C small D, small H, and small C small H. Draw a linkage map illustrating the linkage arrangements among these three genes. Text points to the sample text and reads, DATA ANALYSIS PROBLEMS draw on examples from published research articles and ask students to apply the concepts they learned to analyze real data.

NEW! CONNECTING QUESTIONS help students synthesize concepts from different chapters to understand the big picture. The new questions ensure that students get the bigger picture of the science of genetics and, much like real geneticists, learn to use information from different fields within genetics in problem solving.



Section 8.3

CONNECT

***29.** Red–green color blindness is a human X-linked recessive disorder (see Chapter 4). A young man with a 47,XXY karyotype (Klinefelter syndrome) is color blind. His 46,XY brother is also color blind. Both parents have color vision. In which parent and where in meiosis did the nondisjunction that gave rise to the young man with Klinefelter syndrome take place? Assume that no crossing over took place in prophase I of meiosis (see Chapter 2).

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Description

Text on the left pointing to the sample text reads, NEW! CONNECTING QUESTIONS help students synthesize concepts from different chapters to understand the big picture. The new questions ensure that students get the bigger picture of the science of genetics and, much like real geneticists, learn to use information from different fields within genetics in problem solving. The sample text reads, Section 8 point 3 29. A logo is present below with text that reads, connect. The body text reads as follows, Red–green color blindness is a human X- linked recessive disorder (see Chapter 4). A young man with a 47, X X Y karyotype (Klinefelter syndrome) is color blind. His 46, X Y brother is also color blind. Both parents have color vision. In which parent and where in meiosis did the nondisjunction that gave rise to the young man with Klinefelter syndrome take place? Assume that no crossing over took place in prophase 1 of meiosis (see Chapter 2).

NEW! CONCEPT MAPPING QUESTIONS, available in the book and in SaplingPlus, ask students to build a visual representation of a topic.

Section 2.3

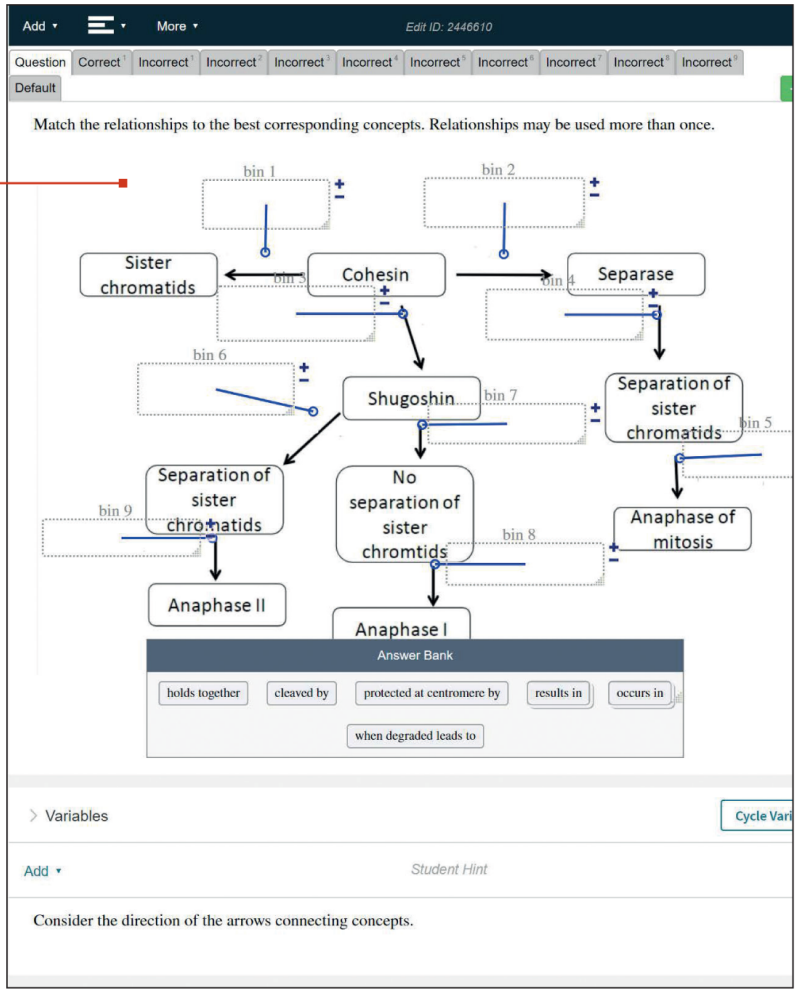
3. Create a concept map that outlines the role of cohesin in the separation of sister chromatids and homologous chromosomes in mitosis and meiosis.

Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

Description

Text on the left pointing to the sample text reads, NEW! CONCEPT MAPPING QUESTIONS, available in the book and in SaplingPlus, ask students to build a visual representation of a topic. The sample text reads, Section 2 point 3, 3. Create a concept map that outlines the role of cohesion in the separation of sister chromatids and homologous chromosomes in mitosis and meiosis.

Concept mapping problems available in SaplingPlus allow students to test their skill through automatically graded exercises.



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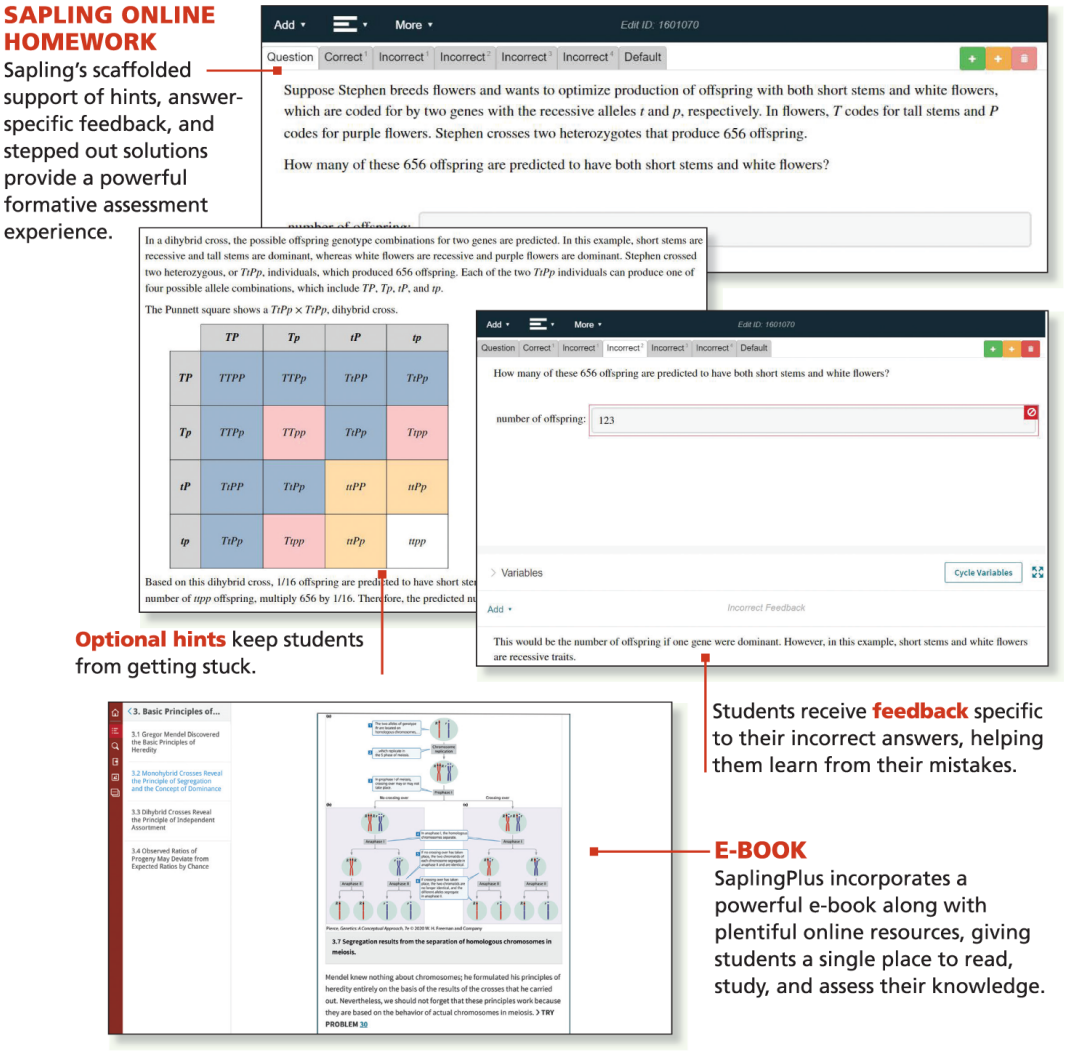
Description
Text on the left pointing to the sample reads, Concept mapping problems available in SaplingPlus allow students to test their skill¬ through automatically graded exercises. It points to a screenshot on the right that shows blank bins (rectangular boxes) labeled 1 through 9 and arrows that link one box to other. Text at the top of example mapping problem reads, Match the relationship to best corresponding concepts. Relationships may be used more than once. Bin 1 corresponds to the arrow from Cohesin to Sister chromatids. Bin 2 corresponds to the arrow from Cohesin to Separase. A third arrow from Cohesin, corresponding to bin 5, points to Shugoshin. An arrow from Separase to Serparation of syster chromatids corresponds to bin 4. An arrow from Separation of sister chromatids to Anaphase of mitosis corresponds to bin 5. An arrow from Shugoshin to Separation of sister chromatids corresponds to bin 6. An arrow from Separation of sister chromatids to corresponds to bin 7. A second arrow from Shugoshin to Anaphase 2 corresponds to bin 9. An arrow from No separation of sister chromatids to Anaphase 1 corresponds to bin 8. The text boxes in an answer bank below read, holds together, cleaved by, protected at centromere by, results in, occurs in, and when degraded leads to. Text at the bottom reads, consider the direction of the arrows connecting concepts. All the bins have a positive and a negative sign button on their top right corners.

SaplingPlus: Tools for Success

SaplingPlus is the online companion to ***Genetics: A Conceptual Approach*** and combines Sapling's acclaimed automatically graded online homework with a powerful e-book and an extensive suite of engaging multimedia learning resources. Problems feature hints for when students get stuck, answer-specific feedback to help

them learn from their mistakes, and solutions to reinforce what they've learned.



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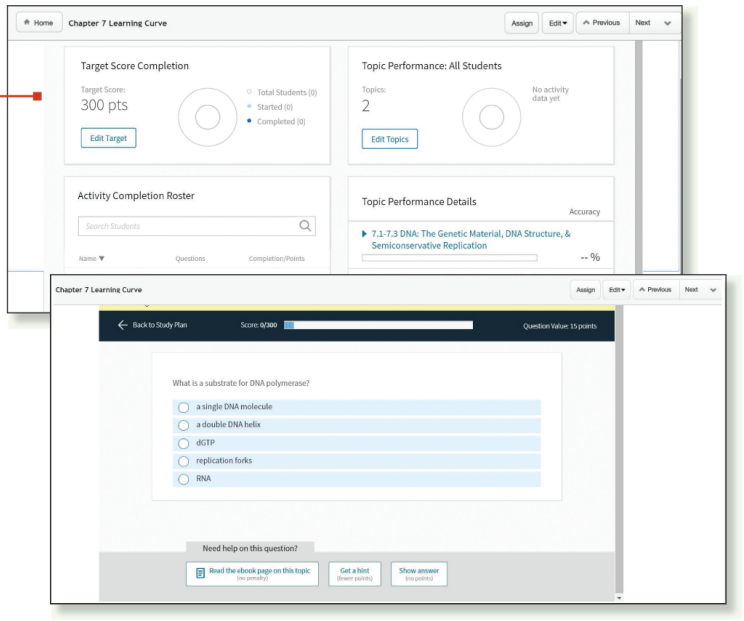
Description

The sample shows a screenshot at the top with a question asking information regarding offspring upon breeding of flowers. Text to its left pointing to the sample reads, SAPLING ONLINE HOMEWORK Sapling's scaffolded support of hints, answer specific feedback, and stepped out solutions provide a powerful formative assessment experience. A screenshot below shows a 4 cross 4 punnett square. Text below reads, Optional hints keep students from getting stuck. A screenshot on the right shows a box to fill the answer.

Text below shows feedback. Text corresponding to this screenshot reads, Students receive feedback specific to their incorrect answers, helping them learn from their mistakes. A screenshot below shows a diagram showing eventual formation of daughter cells. Text corresponding to this screenshot at the right reads, E-BOOK SaplingPlus incorporates a powerful e-book along with plentiful online resources, giving students a single place to read, study, and assess their knowledge.

SaplingPlus also includes tools to help students prepare for class and study for their exams. LearningCurve adaptive quizzing ties back to the e-book and is a great tool to help students learn basic concepts and do assigned readings before coming to lecture. SaplingPlus also includes a wealth of multimedia and problem solving resources to help students make the most of their study time.

LEARNINGCURVE
Put "testing to learn" into action. Based on research, LearningCurve really works: Game-like quizzing motivates students and adapts to their needs based on their performance. It is the perfect tool to get them to engage before class and review after! Additional reporting tools and metrics help teachers get a handle on what their class knows and doesn't know.

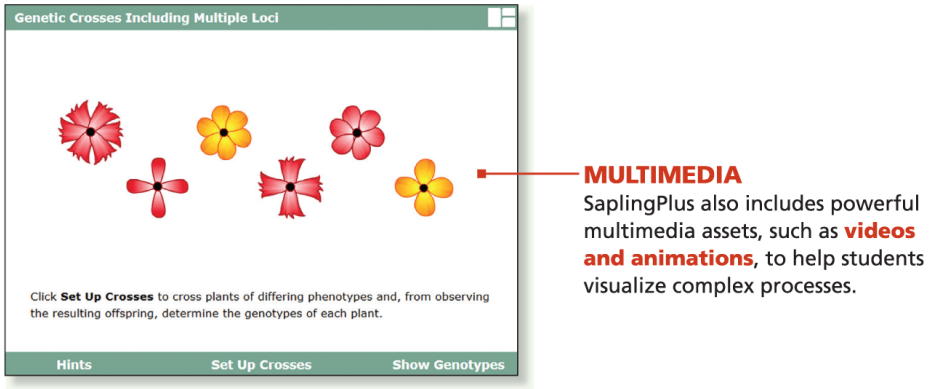


Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

Description

A screenshot shows two columns. The first column below on the left shows target score completion and activity completion roster along with activity data. The second column on the right shows topic performance: all students. It shows number of topics done and topic performance details. Text to the left corresponding to the sample reads, LEARNING CURVE Put “testing to learn” into action. Based on research, LearningCurve really works: Game-like quizzing motivates students and adapts to their needs based on their performance. It is the perfect tool to get them to engage before class and review after! Additional reporting tools and metrics help teachers get a handle on what their class knows and doesn’t know.

A screenshot below shows a question that reads, what is a substrate for D N A polymerase. Below are 5 options preceded by a circular space to click. Text below reads, Need help on this question? Read e book page on this topic, get a hint, and show answer.



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

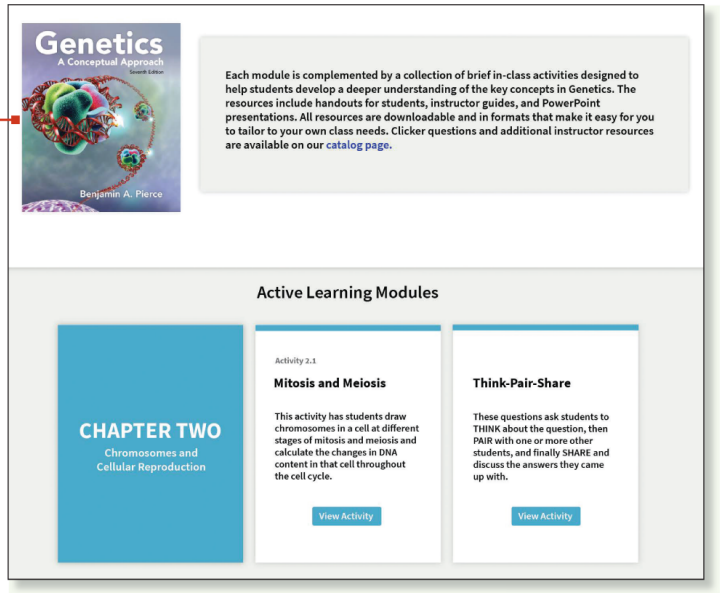
Description

A screenshot shows a heading that reads, genetic crosses including multiple loci. 6 different followers are present below. All the flowers have different shape, different number of petals and different colors. Text below reads, click set up crosses to cross plants of differing phenotypes and, from observing the resulting offspring, determine the genotype of each plant. Buttons on the bottom read, Hints, set up crosses, and show genotypes. Text on the right of the screenshot reads, MULTIMEDIA SaplingPlus also includes powerful multimedia assets, such as videos and animations, to help students visualize complex processes.

Tools for active learning

Genetics: A Conceptual Approach includes support for professors to implement more active learning in the classroom.

NEW! ACTIVE LEARNING HUB with in-class activities is now in SaplingPlus. It contains PowerPoints and worksheets to help you easily implement more active learning in your classroom.



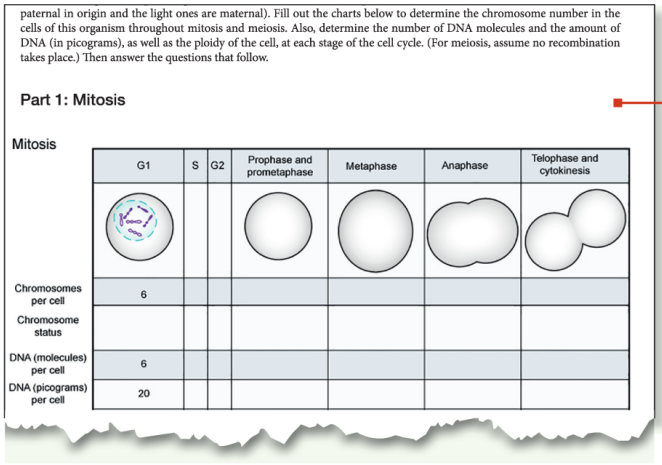
Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

Description

A screenshot on the right shows cover page of a book named Genetics, A Conceptual Approach, Seventh Edition by Benjamin A. Pierce. Text to its right reads, each module is complemented by a collection of brief in class activities designed to help students develop a deeper understanding of the key concepts in genetics. The resources include handouts for students, instructor guides, and PowerPoint presentations. All resources are downloadable and in formats that make it easy for you to tailor to your class needs. Clicker questions and additional instructor resources are available on our catalog page. The screenshot shows active learning modules below. Text on the left of the screenshot reads,

NEW! ACTIVE LEARNING HUB with in-class activities is now in SaplingPlus. It contains PowerPoints and worksheets to help you easily implement more active learning in your classroom.

A panel below shows the heading, chapter 2 chromosomes and cellular reproduction. A panel to its right reads activity 2 point 1 mitosis and meiosis with a view activity button at the bottom. A third panel to the right reads think pair share with a view activity button at the bottom.


paternal in origin and the light ones are maternal). Fill out the charts below to determine the chromosome number in the cells of this organism throughout mitosis and meiosis. Also, determine the number of DNA molecules and the amount of DNA (in picograms), as well as the ploidy of the cell, at each stage of the cell cycle. (For meiosis, assume no recombination takes place.) Then answer the questions that follow.

Part 1: Mitosis

Mitosis

	G1	S	G2	Prophase and prometaphase	Metaphase	Anaphase	Telophase and cytokinesis
Chromosomes per cell	6						
Chromosome status							
DNA (molecules) per cell	6						
DNA (picograms) per cell	20						

Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

Description

A screenshot shows text at the top that reads, paternal in origin and the light ones are maternal). Fill out the charts below to determine the chromosome number in the cells of this organism throughout mitosis and meiosis. Also, determine the number of D N A molecules and the amount of D N A (in picograms), as well as the ploidy of the cell, at each stage of the cell cycle. (For meiosis, assume no recombination take place). Answer the question below.

Part 1: Mitosis. A table below shows 6 columns and 5 rows. The columns headers show different stages of mitosis. The first row contains a cell dividing into two daughter cells gradually from column 1 to column 6. All other rows with different headers are blank.

Text to the right of the screenshot reads, IN-CLASS ACTIVITIES encourage students to apply the concepts that they learn and work, and learn, in groups.

New and Reorganized Content

The seventh edition addresses recent discoveries in genetics corresponding to our ever-changing understanding of inheritance, the molecular nature of genetic information, epigenetics, and genetic evolution. This edition also focuses on updating the new research techniques that have become available to geneticists in the past few years. For example, this edition provides expanded coverage of CRISPR-Cas systems and reorganized and updated content in the chapters on molecular genetic analysis and epigenetics.

New and updated content includes:

- Eight new chapter-opening stories, including "Mapping Fear and Aggression in Dogs" (Chapter 4), "Hominin History in Ancient DNA" (Chapter 12), "Wrecks on the DNA Highway" (Chapter 13), and "Sequencing Earth's Biodiversity" (Chapter 20)
- New Concept Mapping exercises
- New Connecting Concepts problems
- New Learning Objectives in chapter summaries
- Revised section on the cutting edge of genetics (Chapter 1)
- Revised section on binary fission in prokaryotes (Chapter 2)

- Revised and updated coverage of mandatory genetic screening of newborns; updated information on noninvasive prenatal screening; and a new section on genealogies and genetic testing for ancestry (Chapter 6)
- Updated discussion of aneuploidy in humans (Chapter 8)
- Updated discussion of bacterial and archaeal diversity; revised discussion of gene mapping in bacteria; a new section called "Bacterial Defense Mechanisms" and expanded coverage of CRISPR-Cas in bacteria; a new section called "Zika virus"; and an updated discussion of the microbiome (Chapter 9)
- New discussion of topologically associated domains in chromatin structure (Chapter 11)
- Revised and updated discussion of eukaryotic origins of replication (Chapter 12)
- New section on internal mRNA modifications (Chapter 14)
- Updated discussion of variation in translation initiation sites (Chapter 15)
- Revised discussion of transcription factors; new discussion of super enhancers, as well as updated discussion of insulators and regulatory neighborhoods (Chapter 17)
- New discussion of single-cell genomic sequencing to estimate rates of somatic mutations, as well as updated discussion of tautomeric shifts (Chapter 18)
- Extensively updated CRISPR-Cas coverage; new discussion of environmental DNA (eDNA); revised discussion of gene libraries; updated discussion of next-generation and third-generation DNA sequencing methods; updated information on

the CODIS system for DNA fingerprinting; and updated information on gene therapy currently in use (Chapter 19)

- New information on DNA and protein databases; expanded discussion of heat maps; new information on applications of RNA sequencing; and added discussion of the interactome (Chapter 20)
- Extensively reorganized and updated epigenetics content; new discussion of epigenome-wide association studies (Chapter 21)
- New section on mapping gene expression in *Drosophila* embryos; updated information on epigenetic modifications affecting eye development in blind cave fish (Chapter 22)
- Updated cancer statistics; a new discussion of autophagy; and an expanded discussion of the factors that contribute to metastasis (Chapter 23)
- Updated information on the wolves of Isle Royale (Chapter 25)
- A new section called “Interpreting Phylogenetic Trees” (Chapter 26)

Acknowledgments

I am indebted to many people for help with this and previous editions of *Genetics: A Conceptual Approach*. I learned much from my genetics teachers: Ray Canham, who first exposed me to genetics and instilled in me a lifelong love for the subject, and Jeff Mitton, who taught me the art of genetic research. I've learned from the thousands of genetics students who have filled my classes over the past 39 years, first at Connecticut College, then at Baylor University, and now at Southwestern University. Their intelligence, enthusiasm, curiosity, and humor have been a source of motivation and pleasure throughout my professional life. I have also learned from students worldwide who have used earlier editions of this book and kindly shared with me, through emails and phone calls, their thoughts about the book and how it could be improved.

I am grateful for the wonderful colleagues who surround me daily at Southwestern University and whose friendship, advice, and good humor sustain my work. The small classes, close interaction of students and faculty, and integration of teaching and research have made working at Southwestern University personally and professionally rewarding. I thank Edward Burger, President of Southwestern University, and Alisa Gaunder, Dean of the Faculty, for sustaining this supportive academic environment, for their

creativity and inspiration, and for their continued friendship and collegiality.

Writing a modern science textbook requires a team effort, and I have been blessed with an outstanding team at W. H. Freeman and Macmillan Learning. General Manager Susan Winslow has been a champion of this book for a number of years; I value her support, strategic vision, and commitment to education. Sandy Lindelof, Executive Program Director, has been an outstanding leader of the project. Her encouragement, attention to detail, creativity, and great sense of perspective have been tremendous assets. Throughout the revision process, I interacted daily with Senior Development Editor Maria Lokshin, who has been instrumental in shaping this edition. Maria's hard work, passion for excellence, superior knowledge of genetics, great organizational skills, and good humor made working on this edition both rewarding and fun, in spite of a demanding schedule.

Senior Project Manager Vanavan Jayaraman expertly managed the production of this seventh edition. During the production phase of the book, we communicated daily, often late at night. His dedication to excellence in all phases of the production process has been a major factor in making the book a success. I thank Dragonfly Media Group for creating the book's illustrations. Quade Paul (Echo Medical Media) designed the cover image from a concept by Emiko Paul. Thanks to Susan Wein and Lawrence Guerra for coordinating the composition and manufacturing phases of production. Natasha

Wolfe developed the book's design. I thank Jennifer MacMillan and Richard Fox for photo research. Cassandra Korsvik provided excellent advice on many aspects of the book, including organization, content, and pedagogy. Amy Thorne, Cassandra Korsvik, Amber Jonker, Heather Held, and Emiko Paul developed the outstanding media and supplements that accompany the book. Keith Smith, Alexis Cowan, Jared Hale, Amy Terry, Juwen DuBois, Jessica Dade, April Williams, Alyssa Jordan, Kate DeRosa, Heather Khanna developed and reviewed assessment questions. Brenda Grewe and Jared Hale reviewed the Solutions Manual for accuracy.

I am grateful to the Faculty Advisory Board for their invaluable input on the new features of the textbook and new Sapling content. The board includes Eugenia Ribeiro-Hurley (Fordham University), David Kass (Eastern Michigan University), Brian Kreiser (University of Southern Mississippi), Karly Ackermann (South Dakota State University), Kari Loomis (University of Massachusetts), Agata Becalska (MacEwan University), and Amanda Moehring (Western University).

As always, I am grateful to the Macmillan Learning sales representatives, regional managers, and STEM specialists who introduce my book to genetic instructors throughout the world. I have greatly enjoyed working with this sales staff; their expertise, hard work, and great service are responsible for the success of Macmillan books.

A number of colleagues served as reviewers of this book, kindly lending me their technical expertise and teaching experience. Their assistance is gratefully acknowledged. Any remaining errors are entirely my own.

Marlene Tyrrell (my spouse and best friend for 39 years), our children and their spouses (Sarah, Matt, Michael, and Amber), and now my F_2 progeny (Ellie, Beckett, and Caroline) provide love, support, and inspiration for everything I do.

My gratitude goes to the reviewers of this new edition of *Genetics: A Conceptual Approach*:

Preston Aldrich
Benedictine University

Brianne Barker
Drew University

Bruce Bejcek
Western Michigan University

Indrani Bose
Western Carolina University

Nathan J Bowen
Clark Atlanta University

Zenaido Camacho

Western New Mexico University

David B. Carlini
American University

Helen Chamberlin
Ohio State University

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Saint Martin's University

Kavita Oommen
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Chris Petrie
Eastern Florida State College

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Kean University

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Genetics

A Conceptual Approach

CHAPTER 1

Introduction to Genetics

Ansel Adams/National Park Archives at College Park, MD.

A Hopi pueblo on Black Mesa. Albinism, a genetic condition, arises with high frequency among the Hopi people and occupies a special place in the Hopi culture.

Albinism in the Hopis

Rising a thousand feet above the desert floor, Black Mesa dominates the horizon of the Enchanted Desert and provides a familiar

landmark for travelers passing through northeastern Arizona. Black Mesa is not only a prominent geological feature but also, more significantly, the ancestral home of the Hopi Native Americans. Fingers of the mesa reach out into the desert, and alongside or on top of each finger is a Hopi village. Most of the villages are quite small, having only a few dozen inhabitants, but they are incredibly old. One village, Oraibi, has existed on Black Mesa since 1150 C.E. and is the oldest continuously occupied settlement in North America.

In 1900, Aleš Hrdlička, an anthropologist and physician working for the American Museum of Natural History, visited the Hopi villages of Black Mesa and reported a startling discovery. Among the Hopis were 11 white people—not Caucasians but white Hopi Native Americans. These Hopis had a genetic condition known as albinism (**Figure 1.1**).

© The Field Museum, #CSA118. Charles Carpenter.

1.1 Albinism among the Hopi Native Americans. The Hopi girl in the center of this photograph, taken around 1900, displays albinism.

Albinism is caused by a defect in one of the enzymes required to produce melanin, the pigment that darkens our skin, hair, and eyes. People with albinism either don't produce melanin or produce only small amounts of it and, consequently, have white hair, light skin, and no pigment in the irises of their eyes. Melanin normally protects the DNA of skin cells from the damaging effects of ultraviolet radiation in sunlight, and melanin's presence in the developing eye is essential for proper eyesight.

The genetic basis of albinism was first described by the English physician Archibald Garrod, who recognized in 1908 that the condition was inherited as an autosomal recessive trait, meaning that a person must receive two copies of an albino mutation—one

from each parent—to have albinism. In recent years, the molecular nature of the mutations that lead to albinism has been elucidated. Albinism in humans is caused by a mutation in any one of several different genes that control the synthesis and storage of melanin; many different types of mutations can occur at each gene, any one of which may lead to albinism. The form of albinism found among the Hopis is most likely oculocutaneous albinism (albinism affecting the eyes and skin) type 2, caused by a defect in the *OCA2* gene on chromosome 15.

The Hopis are not unique in having people with albinism among the members of their tribe. Albinism is found in almost all human ethnic groups. It is described in ancient writings and has probably been present since humankind's beginnings. What is unique about the Hopis is the high frequency of albinism in their population. In most human groups, albinism is rare, present in only about 1 in 20,000 individuals. In the villages on Black Mesa, it reaches a frequency of 1 in 200, a hundred times higher than in many other populations.

Why is albinism so frequent among the Hopis? The answer to this question is not completely known, but geneticists who have studied albinism in the Hopis speculate that the high frequency of the albino mutation is related to the special place that albinism occupied in the Hopi culture. For much of their history, the Hopis considered members of their tribe with albinism to be important

and special. Having a number of people with albinism in one's village was considered a good sign, a symbol that the people of the village contained particularly pure Hopi blood. Members of the tribe with albinism performed in Hopi ceremonies and held positions of leadership, often as chiefs, healers, and religious leaders.

Hopis with albinism were also given special treatment in everyday activities. The Hopis have farmed small garden plots at the foot of Black Mesa for centuries. Every day throughout the growing season, the men of the tribe trekked to the base of Black Mesa and spent much of the day in the bright southwestern sunlight tending gardens of corn and vegetables. With little or no melanin pigment in their skin, people with albinism are extremely susceptible to sunburn and have an increased incidence of skin cancer when exposed to the sun. Furthermore, many don't see well in bright sunlight. Therefore, the male Hopis with albinism were excused from farming and allowed to remain behind in the village with the women of the tribe, performing other duties.

Throughout the growing season, the men with albinism were the only male members of the tribe in the village with the women during the day, and thus they may have enjoyed a mating advantage, which helped pass on their albino genes. In addition, the special considerations given to Hopis with albinism allowed them to avoid the detrimental effects of albinism—increased skin cancer and poor eyesight. The small size of the Hopi tribe probably also played a

role by allowing chance to increase the frequency of the albino mutation. Regardless of the factors that led to the high frequency of albinism, the Hopis clearly respected and valued the members of their tribe who possessed this particular trait. Unfortunately, people with genetic conditions in many societies are often subject to discrimination and prejudice. **› TRY PROBLEMS 1 AND 26**

THINK-PAIR-SHARE

- Albinism occupied a special place in the Hopi culture; individuals who possessed this trait were valued by members of the tribe. What are some examples of genetic traits that, in contrast, sometimes result in discrimination and prejudice?
- Albinism in humans can be caused by mutations in any one of several different genes. This situation, in which the same phenotype may result from variation in several different genes, is referred to as genetic heterogeneity. Is genetic heterogeneity common? Are most genetic traits in humans the result of variation in a single gene, or are there many genetic traits that result from variation in several genes, as albinism does?

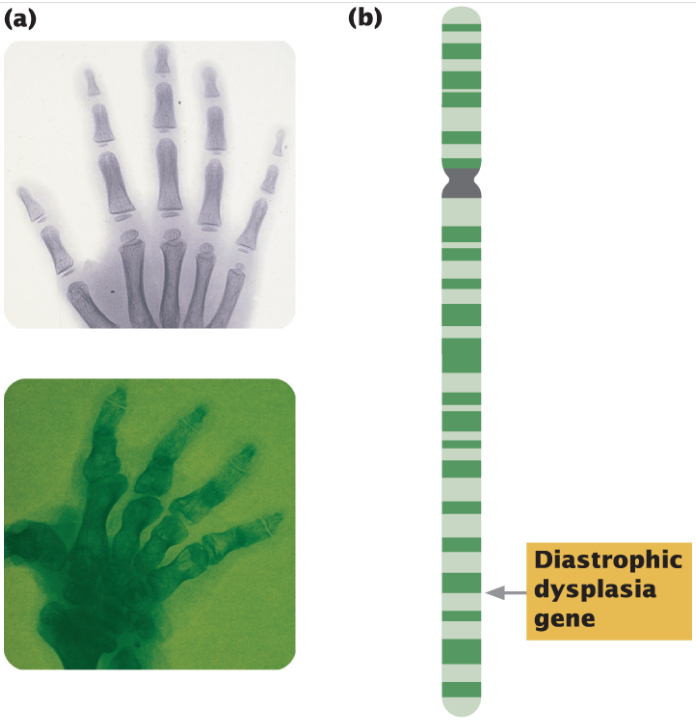
Genetics is the study of heredity, how inherited variation is encoded, replicated, and expressed, and how it evolves over time. This definition encompasses the three major subdivisions of genetics: transmission genetics, molecular genetics, and population genetics. The study of heredity and inherited variation is one of the most rapidly advancing areas of science, with important new discoveries reported every month. Look at almost any major news source, and chances are that you will see articles related to genetics: on the sequencing of new genomes, such as those of the sunflower, barley, the axolotl, and Darwin's finches; on the discovery

of genes that affect major diseases, including amyotrophic lateral sclerosis (ALS), depression, and cancer; on analyses of DNA from long-extinct organisms, such as a 700,000-year-old Pleistocene horse; or on the identification of genes that affect birth weight, skin pigmentation, height, and learning ability in humans. Even among advertisements, you are likely to see ads for genetic testing to determine a person's ancestry or the pedigree of your dog. These new findings and applications of genetics often have significant economic and ethical implications, making the study of genetics relevant, timely, and interesting.

This chapter introduces you to genetics and reviews some concepts that you may have encountered in your general biology course. We begin by considering the importance of genetics to each of us, to society, and to students of biology. We then turn to the history of genetics and how the field as a whole developed. The final part of the chapter presents some fundamental terms and principles of genetics that are used throughout the book.

1.1 Genetics Is Important to Us Individually, to Society, and to the Study of Biology

Albinism among the Hopis illustrates the important role that genes play in our lives. This one genetic alteration, among the 20,000 genes that humans possess, completely changes the life of a Hopi who possesses it. It alters his or her occupation, role in Hopi society, and relations with other members of the tribe. We all possess genes that influence our lives in significant ways. Genes affect our height, weight, hair color, and skin pigmentation. They affect our susceptibility to many diseases and disorders (Figure 1.2) and even contribute to our intelligence and personality. Genes are fundamental to who and what we are, but they alone do not define us. Many factors—genetic and environmental—help determine our traits. A fundamental problem in genetics is separating the genetic and environmental influences on individual variation.



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company
Part a: (top) Biophoto Associates/Science Source; (bottom) Reprinted from Cell, 78(6) Johanna Hästbacka, et al., The diastrophic dysplasia gene encodes a novel sulfate transporter: Positional cloning by fine-structure linkage disequilibrium mapping, *Cell* 78(6): 1073–1087, © 1994 with permission from Elsevier. Permission conveyed through Copyright Clearance Center, Inc. Courtesy of Prof. Eric Lander, Whitehead Institute, MIT.

1.2 Genes influence susceptibility to many diseases and disorders. (a) An X-ray of the hand of a person suffering from diastrophic dysplasia (bottom), a hereditary growth disorder that results in curved bones, short limbs, and hand deformities, compared with an X-ray of a normal hand (top). (b) Diastrophic dysplasia is due to a defect in the *SLC26A2* gene on chromosome 5.

Description

The x-ray pictures on the left show a normal hand with straight fingers on the top. The x-ray below shows the hand of a person with diastrophic dysplasia. This hand has curvy fingers. The drawing “b” on the right shows chromosome 5. The diastrophic dysplasia

gene is located on the long arm of this. The chromosome has multiple bands of varying thickness all throughout.

Although the science of genetics is relatively new compared with sciences such as astronomy and chemistry, people have understood the hereditary nature of traits and practiced genetics for thousands of years. The rise of agriculture began when people started to apply genetic principles to the domestication of plants and animals. Today, the major crops and animals used in agriculture are quite different from their wild progenitors, having undergone extensive genetic alteration that increased their yields and provided many desirable traits, such as disease and pest resistance, special nutritional qualities, and characteristics that facilitate harvest. The Green Revolution, which expanded food production throughout the world in the 1950s and 1960s, relied heavily on the application of genetic methods and principles (**Figure 1.3**). Today, genetically engineered corn, soybeans, canola (rapeseed), squash, sugar beets, and other crops constitute a significant proportion of all the food produced worldwide.

Left: Bettmann/Getty Images. right: IRRI.

1.3 In the Green Revolution, genetic techniques were used to develop new high-yielding strains of crops. (Left) Norman Borlaug, a leader in the development of new varieties of wheat, which led to the Green Revolution. Borlaug was awarded the Nobel Peace Prize in 1970. (Right) Modern, high-yielding rice plant (left) and traditional rice plant (right).

Description
The first photo shows Norman Borlaug writing in a notebook while standing in a rice field. The second photo shows a small, traditional rice plant with tightly packed, shorter stems and spikelets next to the tall, high-yielding modern rice plant with longer stems and spikelets.

The pharmaceutical industry is another area in which genetics plays an important role. Numerous drugs and food additives are synthesized by fungi and bacteria that have been genetically manipulated to make them efficient producers of these substances. The biotechnology industry employs molecular genetic techniques

to develop and mass-produce substances of commercial value. Antimalarial drugs, growth hormone, insulin, clotting factor, antiviral drugs, enzymes, antibiotics, vaccines, and many other compounds are now produced commercially by genetically engineered bacteria and other organisms (Figure 1.4). Genetics has also been used to generate bacterial strains that remove minerals from ore, break down toxic chemicals, and help produce biofuels.

Traimak_Ivan/Getty Images.

1.4 The biotechnology industry uses molecular genetic methods to produce substances of economic value.

Genetics also plays a critical role in medicine. Physicians recognize that many diseases and disorders have a hereditary component, including rare genetic disorders such as sickle-cell anemia and Huntington disease, as well as many common diseases such as asthma, diabetes, and hypertension. Advances in genetics have resulted in important insights into the nature of diseases such as cancer and in the development of diagnostic tests, including tests

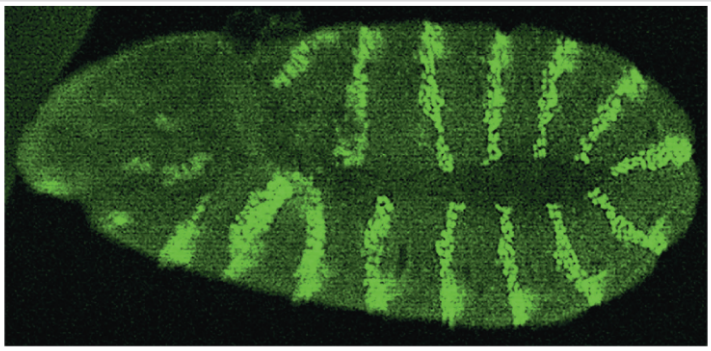
that identify disease-causing mutations as well as pathogens. Genomic data are helping to usher in the era of personalized medicine. Rapid, low-cost sequencing methods now allow us to obtain a person's complete genome sequence, which provides important information about that person's susceptibilities to diseases and likely responses to particular treatments. And gene therapy—the direct alteration of genes to treat human diseases—has now been administered to thousands of patients, although its use is still experimental and limited.

THINK-PAIR-SHARE Question 1

The Role of Genetics in Biology

Although an understanding of genetics is important to all people, it is critical to the student of biology. Genetics provides one of biology's unifying principles: all organisms use genetic systems that have a number of features in common. Genetics also undergirds the study of many other biological disciplines. Evolution, for example, is genetic change taking place over time, so the study of evolution requires an understanding of genetics. Developmental biology relies heavily on genetics: tissues and organs develop through the regulated expression of genes (**Figure 1.5**). Even such fields as taxonomy, ecology, and animal behavior are making increasing use of genetic methods. The study of almost any field of biology or

medicine is incomplete without a thorough understanding of genes and genetic methods.



Steven Paddock.

1.5 The key to development lies in the regulation of gene expression. This early fruit-fly embryo illustrates the localized expression of the *engrailed* gene, which helps determine the development of body segments in the adult fly.

Genetic Diversity and Evolution

Life on Earth exists in a tremendous array of forms and features, occupying almost every conceivable environment. Life is also characterized by adaptation: many organisms are exquisitely suited to the environment in which they are found. The history of life is a chronicle of new forms of life emerging, old forms disappearing, and existing forms changing.

Despite their tremendous diversity, living organisms have an important feature in common: all use similar genetic systems. The complete set of genetic instructions for any organism is its **genome**, and all genomes are encoded in nucleic acids—either DNA or RNA.

The coding system for genomic information is also common to all life: genetic instructions are in the same format and, with rare exceptions, the code words are identical. Likewise, the process by which genetic information is copied and decoded is remarkably similar for all forms of life. These common features suggest that all life on Earth evolved from the same primordial ancestor, which arose between 3.5 billion and 4 billion years ago. Biologist Richard Dawkins describes life as a river of DNA that runs through time, connecting all organisms past and present.

That all organisms have similar genetic systems means that the study of one organism's genes reveals principles that apply to other organisms. Investigations of how bacterial DNA is replicated (copied), for example, provide information that applies to the replication of human DNA. It also means that genes can often function in foreign cells, which makes genetic engineering possible. Unfortunately, the similarity of genetic systems is also the basis for diseases such as AIDS (acquired immune deficiency syndrome), in which viral genes are able to function—sometimes with alarming efficiency—in human cells.

Life's diversity and adaptations are products of evolution, which is simply genetic change over time. Evolution is a two-step process: first, inherited differences arise randomly, and then the proportion of individuals with particular differences increases or decreases. Genetic variation is therefore the foundation of all evolutionary change and is ultimately the basis of all life as we know it.

Techniques of molecular genetics are now routinely used to decipher evolutionary relationships among organisms; for example, analysis of DNA isolated from Neanderthal fossils has yielded new information concerning the relationship between Neanderthals and modern humans, demonstrating that Neanderthals and the ancestors of modern humans interbred some 30,000 to 40,000 years ago. Genetics and the study of genetic variation are critical to understanding the past, present, and future of life. **› TRY PROBLEM 17**

THINK-PAIR-SHARE Question 2

CONCEPTS

Our genes affect many of our physical features as well as our susceptibility to many diseases and disorders. Genetics contributes to advances in agriculture, pharmaceuticals, and medicine and is fundamental to modern biology. All organisms use similar genetic systems, and genetic variation is the foundation of the diversity of all life.

✔ CONCEPT CHECK 1

What are some of the implications of all organisms having similar genetic systems?

a. That all life forms are genetically related
b. That research findings on one organism's gene function can often be applied to other organisms
c. That genes from one organism can often function in another organism
d. All of the above

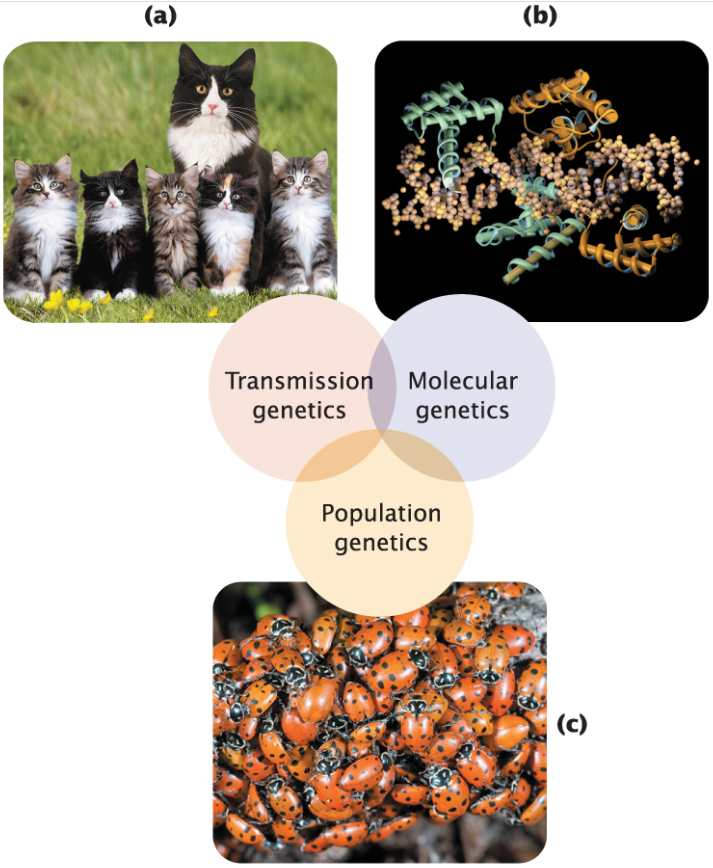
DNA in the Biosphere

Each DNA molecule is very small, but because all cells contain genetic information, there is a tremendous amount of DNA in the world. Scientists estimate that the total amount of DNA in the biosphere is 5.3×10^{31} megabase pairs (millions of base pairs), altogether weighing some 50 billion tons. Storing the information content of the world's DNA would require 10^{21} computers, each with the average storage capacity of the world's four most powerful supercomputers.

Scientists are now cataloging and measuring the world's biodiversity through analysis of DNA. For example, researchers aboard the ship *Tara* surveyed the world's oceans for organisms by isolating DNA from seawater during a three-and-a-half-year voyage. They collected 35,000 seawater samples and extracted DNA from each. The DNA was then sequenced and analyzed, revealing the presence of 150,000 genetically distinct types of eukaryotes. Most of these eukaryotes were newly discovered single-celled organisms. The researchers also detected 5000 viruses, only 39 of which were previously known to science. In an effort named the Earth BioGenome Project (EBP), scientists have recently proposed sequencing the genomes of 1.5 million known eukaryotic species on Earth. (See the Suggested Readings for this chapter in your SaplingPlus for the reference to these studies and many others mentioned in the book.)

Divisions of Genetics

The study of genetics consists of three major subdisciplines: transmission genetics, molecular genetics, and population genetics (Figure 1.6). Also known as classical genetics, **transmission genetics** encompasses the basic principles of heredity and how traits are passed from one generation to the next. This subdiscipline addresses the relation between chromosomes and heredity, the arrangement of genes on chromosomes, and gene mapping. Here, the focus is on the individual organism—how an individual inherits its genetic makeup and how it passes its genes to the next generation.



Part a: Juniors Bildarchiv/Alamy Stock Photo. Part b: Martin McCarthy/Getty Images. Part c: Stuart Wilson/Science Source.

1.6 Genetics can be divided into three major subdisciplines.

Description
The photo “a” on the top left representing transmission genetics shows an adult tuxedo cat with 5 kittens, 2 looking like tuxedo cats and 3 looking like tabbies. The photo “b” on the top right representing molecular genetics shows a 3-D ribbon model of a biomolecule with helical D N A at the center surrounded by ribbons of green and yellow color. The photo “c” below representing population genetics shows a flock of ladybugs. A Venn diagram at the center shows overlapping between transmission genetics, molecular genetics, and population genetics.

Molecular genetics concerns the chemical nature of the gene itself: how genetic information is encoded, replicated, and expressed. It includes the cellular processes of replication, transcription, and translation (by which genetic information is transferred from one molecule to another) and gene regulation (the processes that control the expression of genetic information). The focus in molecular genetics is the gene and its structure, organization, and function.

Population genetics explores the genetic composition of populations (groups of individuals of the same species) and how that composition changes geographically and with the passage of time. Because evolution is genetic change, population genetics is fundamentally the study of evolution. The focus of population genetics is the group of genes found in a population.

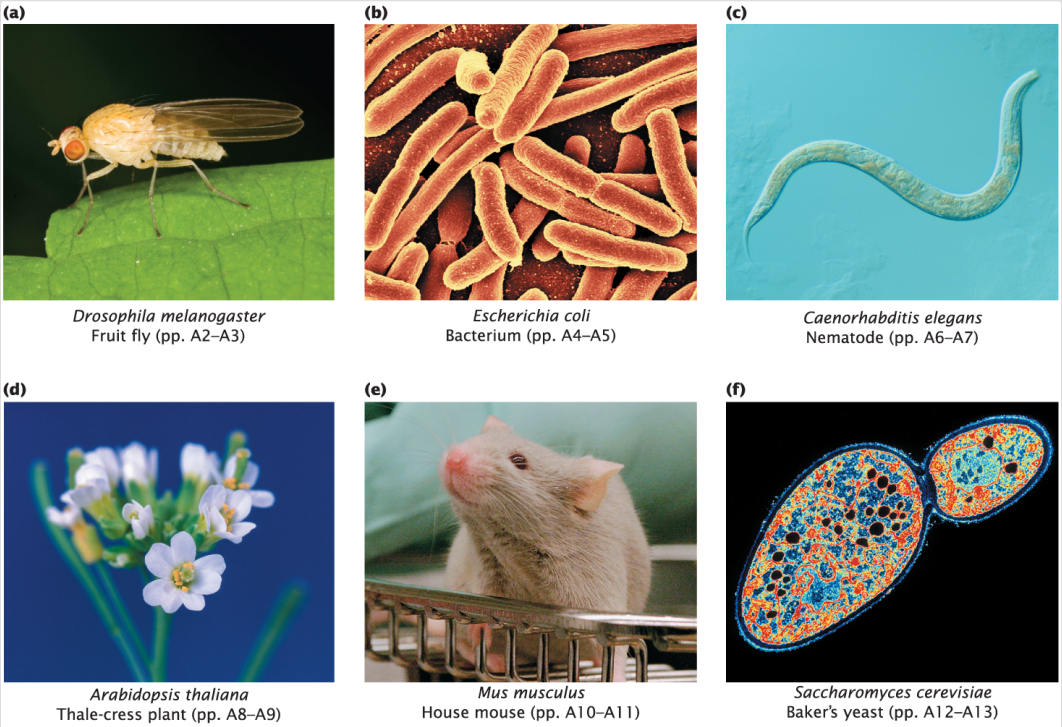
Division of the study of genetics into these three subdisciplines is convenient and traditional, but we should recognize not only that

they overlap but also that each one can be further divided into a number of more specialized fields, such as chromosomal genetics, biochemical genetics, and quantitative genetics. Alternatively, genetic studies can be subdivided by organism (fruit fly, corn, or bacterial genetics), and each of these organisms may be studied at the levels of transmission, molecular, and population genetics. Modern genetics is an extremely broad field, encompassing many interrelated subdisciplines and specializations. › **TRY** PROBLEM 18

Model Genetic Organisms

Through the years, genetic studies have been conducted on thousands of different species, including almost all major groups of bacteria, fungi, protists, plants, and animals. Nevertheless, a few species have emerged as **model genetic organisms**—organisms that are widely used in genetic research and that can serve as models for the genetic systems of other organisms, like humans, which, for various reasons, may be more difficult to study. Genetic models typically have characteristics that make them particularly useful for genetic analysis and about which a tremendous amount of genetic information has accumulated. Six model organisms that have been the subject of intensive genetic study are *Drosophila melanogaster*, a species of fruit fly; *Escherichia coli*, a bacterium present in the gut of humans and other mammals; *Caenorhabditis elegans*, a soil-dwelling nematode (roundworm); *Arabidopsis thaliana*, the thale-cress plant;

Mus musculus, the house mouse; and *Saccharomyces cerevisiae*, baker's yeast (Figure 1.7). The life cycles and genetic characteristics of these model genetic organisms are described in more detail in the Reference Guide to Model Genetic Organisms located at the end of this book (pp. A1–A13). This Reference Guide will be a useful resource as you encounter these organisms throughout the book.



Part a: © Alfred Schauhuber/ImageBROKER/Alamy Stock Photo. Part b: Pasieka/Science Source. Part c: Sinclair Stammers/Science Source. Part d: Peggy Greb/ARS/USDA. Part e: AP Photo/Joel Page. Part f: Biophoto Associates/Science Source.

1.7 Model genetic organisms are species with features that make them useful for genetic analysis.

Description

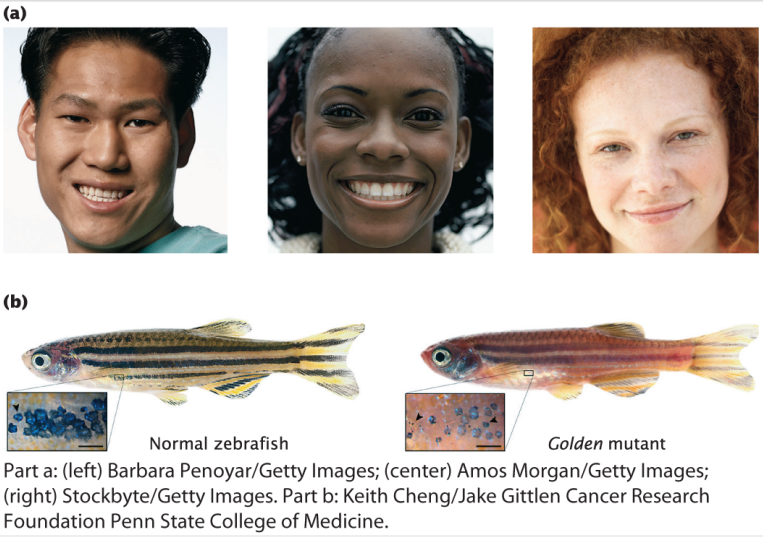
Photo "a" on top left shows Drosophila melanogaster (Fruit fly) sitting over a leaf. Photo "b" in top center shows several rod shaped Escherichia coli Bacterium. Photo "c" in top right shows thin tube shaped Caenorhabditis elegans (Nematode). Photo "d" on bottom left shows Arabidopsis thaliana (Thale-cress plant). Photo "e" on the bottom center shows Mus musculus (House mouse). Photo "f" on bottom right shows Saccharomyces

cerevisiae (Baker's yeast), which has two oval shaped cells attached. The one on the right is smaller as compared to the one on the left.

At first glance, these lowly and sometimes unappreciated creatures might seem unlikely candidates for model genetic organisms. However, all possess life cycles and traits that make them particularly suitable for genetic study, including a short generation time, large but manageable numbers of progeny, adaptability to a laboratory environment, and the ability to be housed and propagated inexpensively. Other species that are frequently the subjects of genetic research and considered genetic models include *Neurospora crassa* (bread mold), *Zea mays* (corn), *Danio rerio* (zebrafish), and *Xenopus laevis* (clawed frog). Although not generally considered a model genetic organism, *Homo sapiens* has also been subjected to intensive genetic scrutiny; special techniques for the genetic analysis of humans are discussed in Chapter 6.

The value of model genetic organisms is illustrated by the use of zebrafish to identify genes that affect skin pigmentation in humans. For many years, geneticists recognized that differences in pigmentation among human ethnic groups were genetic (**Figure 1.8a**), but the genes causing these differences were largely unknown. The zebrafish has become an important model in genetic studies because it is a small vertebrate that produces many offspring and is easy to rear in the laboratory. The mutant zebrafish called *golden* has light pigmentation due to the presence of fewer, smaller, and less

dense pigment-containing structures called melanosomes in its cells (Figure 1.8b).



Part a: (left) Barbara Penoyar/Getty Images; (center) Amos Morgan/Getty Images; (right) Stockbyte/Getty Images. Part b: Keith Cheng/Jake Gittlen Cancer Research Foundation Penn State College of Medicine.

1.8 The zebrafish, a model genetic organism, has been instrumental in helping identify genes encoding pigmentation differences among humans. (a) Humans differ in degree of skin pigmentation. (b) The zebrafish *golden* mutation is caused by a gene that controls the amount of melanin in melanosomes.

Description

Photo a shows an Asian man with a moderate skin tone and dark hair, an African girl with dark skin and hair, and a Scotch-Irish woman with fair skin and freckles. Photo b shows a normal zebra fish and a golden mutant zebra fish; both vary in color. A magnified view of the fish scales show the normal zebra fish with tightly clustered dark scales and an underlying yellow skin tone and the golden mutant with loosely clustered scales and an underlying orange skin tone.

Keith Cheng and his colleagues hypothesized that light skin in humans might result from a mutation that is similar to the *golden* mutation in zebrafish. Taking advantage of the ease with which zebrafish can be manipulated in the laboratory, they isolated and

sequenced the gene responsible for the *golden* mutation and found that it encodes a protein that takes part in calcium uptake by melanosomes. They then searched a database of all known human genes and found a similar gene called *SLC24A5*, which encodes a protein that has the same function in human cells. When they examined human populations, they found that light-skinned Europeans often possess one form of this gene, whereas darker-skinned Africans, East Asians, and Native Americans usually possess a different form. Many other genes also affect pigmentation in humans, as illustrated by the mutations in the *OCA2* gene that produce albinism among the Hopis (discussed in the introduction to this chapter). Nevertheless, *SLC24A5* appears to be responsible for 24% to 38% of the differences in pigmentation between Africans and Europeans.

This example illustrates the power of model organisms in genetic research. However, we should not forget that all organisms possess unique characteristics and that the genetics of model organisms do not always accurately reflect the genetic systems of other organisms.

CONCEPTS

The three major divisions of genetics are transmission genetics, molecular genetics, and population genetics. Transmission genetics examines the principles of heredity; molecular genetics deals with the gene and the cellular processes by which genetic information is transferred and expressed; population genetics concerns the genetic composition of groups of organisms and how that composition changes geographically and over time. Model genetic

organisms are species that have received special emphasis in genetic research; they have characteristics that make them useful for genetic analysis.

✔ CONCEPT CHECK 2

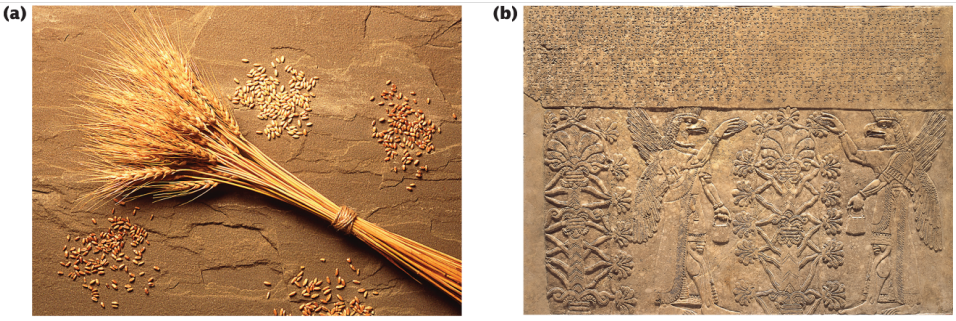
Would the horse make a good model genetic organism? Why or why not?

1.2 Humans Have Been Using Genetic Techniques for Thousands of Years

Although the science of genetics is young—almost entirely a product of the past 120 years or so—people have been using genetic principles for thousands of years.

The Early Use and Understanding of Heredity

The first evidence that people understood and applied the principles of heredity in earlier times is found in the domestication of plants and animals, which began between approximately 10,000 and 12,000 years ago; early farming villages appeared in the Middle East between 11,000 and 11,500 years ago. The first domesticated organisms included wheat, peas, lentils, barley, dogs, goats, and sheep (Figure 1.9a). By 4000 years ago, genetic techniques of selective breeding were already in use in the Middle East. The Assyrians and Babylonians developed several hundred varieties of date palms that differed in fruit size, color, taste, and time of ripening (Figure 1.9b). Other crops and domesticated animals were developed by cultures in Asia, Africa, and the Americas in the same period.



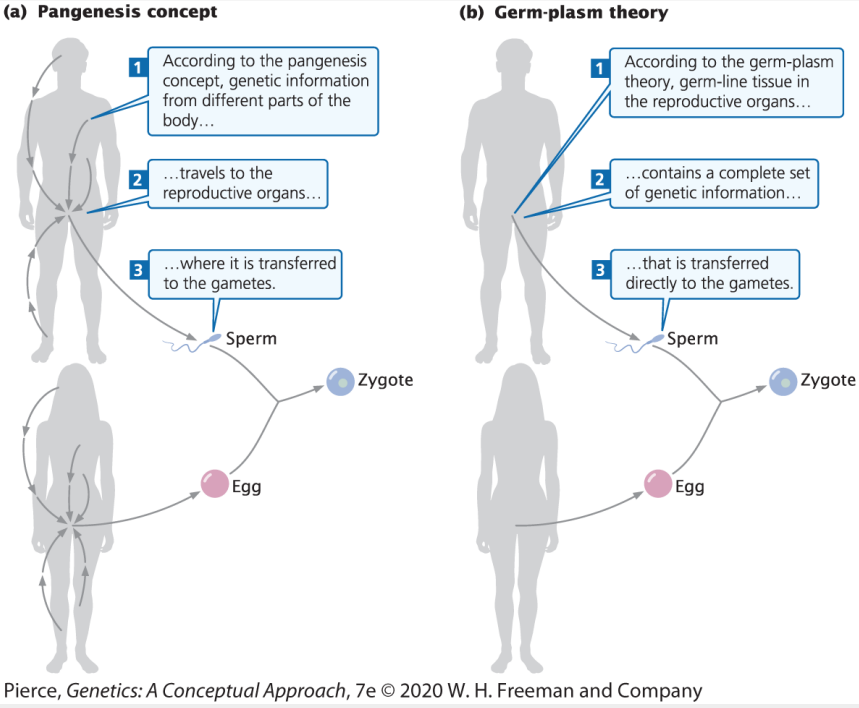
Part a: Scott Bauer/ARS/USDA. Part b: Lower register: Image copyright © The Metropolitan Museum of Art. Image source: Art Resource, NY.

1.9 Ancient peoples practiced genetic techniques in agriculture. (a) Modern wheat, with larger and more numerous seeds that do not scatter before harvest, was produced by interbreeding at least three different wild species. (b) Assyrian bas-relief sculpture showing artificial pollination of date palms at the time of King Ashurnasirpal II, who reigned from 883 to 859 B.C.E.

Description
The photo shows a small sheaf of wheat with grains alongside. The sculpture depicts two mystical figures facilitating artificial pollination of date palms.

Ancient writings demonstrate that early humans were also aware of their own heredity. Hindu sacred writings dating to 2000 years ago suggest that many traits are inherited from the father and that differences between siblings are produced by the mother. The Talmud—the Jewish book of religious laws based on oral traditions dating back thousands of—years, presents an uncannily accurate understanding of the inheritance of hemophilia. It directs that if a woman bears two sons who die of bleeding after circumcision any additional sons that she bears should not be circumcised, nor should the sons of her sisters be circumcised. This advice accurately corresponds to the X-linked pattern of inheritance of hemophilia (discussed further in Chapter 6).

Some early concepts of heredity were incorrect but reflect human interest in heredity and our attempts to explain the inheritance of traits. The ancient Greeks gave careful consideration to human reproduction and heredity. Greek philosophers developed the concept of **pangenesis**, in which each part of the body contains genetic information for that particular part. Specific particles, later called gemmules, carry information from various parts of the body to the reproductive organs, from which they are passed to the embryo at the moment of conception (Figure 1.10a). Although incorrect, the concept of pangenesis was highly influential and persisted until the late 1800s.



1.10 Pangenesis, an early concept of inheritance, compared with the modern germ-plasm theory.

Description

Diagram “a” on the left shows the pangenesis concept. Four arrows from different body parts are marked towards the reproductive organs in man and five arrows from different parts are marked towards reproductive organs in woman. An arrow extends from both man and woman’s reproductive organs points to sperm and egg, respectively. Then, zygote is formed. Three captions read, according to the pangenesis concept, genetic information from different parts of the body, indicated by arrows showing movement from different parts of the body, travels to the reproductive organs, where the information is transferred to the sperm or egg.

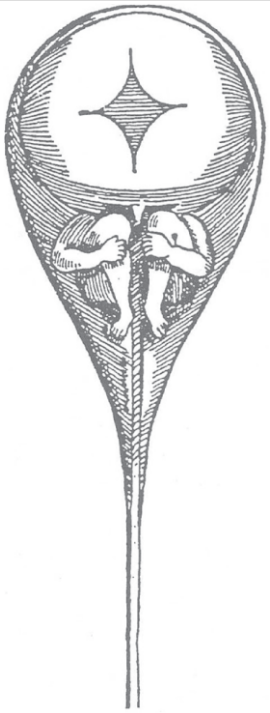
Diagram “b” on the right shows the germplasm concept. An arrow extends from both man and woman’s reproductive organs to sperm and egg, respectively. Then, zygote is formed. Three captions read, according to the germplasm theory, germ-line tissue in the reproductive organs contain a complete set of genetic information that is transferred directly to the sperm and egg.

The concept of pangenesis led the ancient Greeks to propose the notion of the **inheritance of acquired characteristics**, according to which traits acquired in a person’s lifetime become incorporated into that person’s hereditary information and are passed on to offspring; for example, they proposed that people who developed musical ability through diligent study would produce children who were innately endowed with musical ability. Jean-Baptiste Lamarck (1744–1829) was a proponent of this idea and incorporated it into his theory of biological change. The notion of the inheritance of acquired characteristics is also no longer accepted, but it remained popular through the twentieth century.

Although the ancient Romans contributed little to an understanding of human heredity, they successfully developed a number of techniques for animal and plant breeding; their techniques were

based on trial and error rather than any general concept of heredity. Little new information was added to the understanding of genetics in the next thousand years.

Additional developments in our understanding of heredity occurred during the seventeenth century. Dutch eyeglass makers began to put together simple microscopes in the late 1500s, enabling Robert Hooke (1635–1703) to discover cells in 1665. Microscopes provided naturalists with new and exciting vistas on life. Perhaps it was excessive enthusiasm for this new world of the very small that gave rise to the idea of **preformationism**: that inside the egg or sperm there exists a fully formed miniature adult, a *homunculus*, which simply enlarges in the course of development (**Figure 1.11**). Preformationism meant that all traits were inherited from only one parent—from the father if the homunculus was in the sperm or from the mother if it was in the egg. Although many observations suggested that offspring possess a mixture of traits from both parents, preformationism remained a popular concept throughout much of the seventeenth and eighteenth centuries.



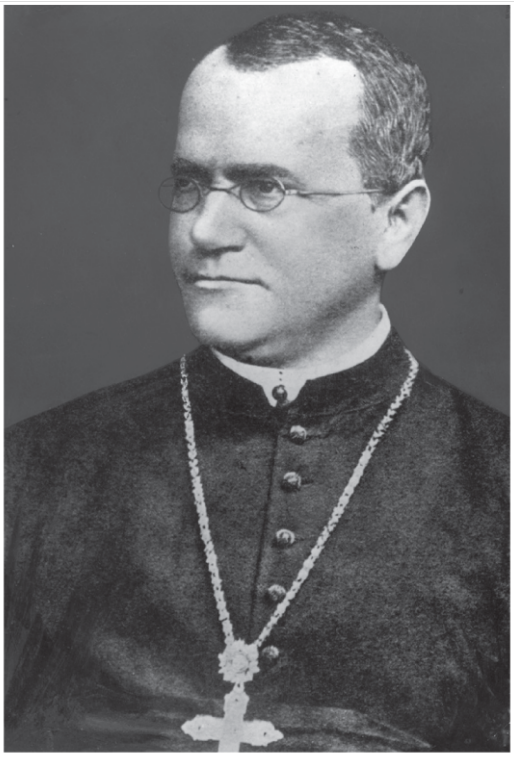
Science Source.

1.11 Preformationists in the seventeenth and eighteenth centuries believed that sperm or eggs contained a fully formed human (the homunculus). Shown here is a drawing of a homunculus inside a sperm.

Another early notion of heredity was **blending inheritance**, which proposed that the traits of offspring are a blend, or mixture, of parental traits. This idea suggested that the genetic material itself blends, much as blue and yellow pigments blend to make green paint; it also suggested that after having been blended, genetic differences could not be separated in future generations, just as green paint cannot be separated into blue and yellow pigments. Some traits do *appear* to exhibit blending inheritance; however, we realize today that individual genes do not blend.

The Rise of the Science of Genetics

In 1676, Nehemiah Grew (1641–1712) reported that plants reproduce sexually. With this information, a number of botanists began to experiment with crossing plants and creating hybrids, including Gregor Mendel (1822–1884; Figure 1.12), who went on to discover the basic principles of heredity. Mendel's conclusions, which were not widely known in the scientific community until 35 years after their publication, laid the foundation for our modern understanding of heredity, and he is generally recognized today as the father of genetics.



Hulton Archive/Getty Images.

1.12 Gregor Mendel is the father of modern genetics. Mendel first discovered the principles of heredity by crossing different varieties of pea plants and analyzing

the transmission of traits in subsequent generations.

Developments in cytology (the study of cells) in the 1800s had a strong influence on genetics. Robert Brown (1773–1858) described the cell nucleus in 1833. Building on the work of others, Matthias Jacob Schleiden (1804–1881) and Theodor Schwann (1810–1882) proposed the concept that came to be known as the **cell theory** in 1839. According to this theory, all life is composed of cells, cells arise only from preexisting cells, and the cell is the fundamental unit of structure and function in living organisms. Biologists interested in heredity began to examine cells to see what took place in the course of cell reproduction. Walther Flemming (1843–1905) observed the division of chromosomes in 1879 and published a superb description of mitosis. By 1885, biologists generally recognized that the cell nucleus contains the hereditary information.

Charles Darwin (1809–1882), one of the most influential biologists of the nineteenth century, put forth the theory of evolution through natural selection and published his ideas in *On the Origin of Species* in 1859. Darwin recognized that heredity was fundamental to evolution, and he conducted extensive genetic crosses with pigeons and other organisms. He never understood the nature of inheritance, however, and this lack of understanding was a major omission in his theory of evolution.

In the last half of the nineteenth century, cytologists demonstrated that the nucleus had a role in fertilization. Near the close of that

century, August Weismann (1834–1914) finally laid to rest the notion of the inheritance of acquired characteristics. He cut off the tails of mice for 22 consecutive generations and showed that the tail length in descendants remained stubbornly long. Weismann proposed the **germ-plasm theory**, which holds that the cells in the reproductive organs carry a complete set of genetic information that is passed to the egg and sperm (**Figure 1.10b**).

The year 1900 was a watershed in the history of genetics. Gregor Mendel's pivotal 1866 publication on experiments with pea plants, which revealed the principles of heredity (considered in more detail in Chapter 3), was rediscovered. Once the significance of his conclusions was recognized, other biologists immediately began to conduct similar genetic studies on mice, chickens, and other organisms. The results of these investigations showed that many traits indeed follow Mendel's rules. Some of the early concepts of heredity are summarized in **Table 1.1**.

TABLE 1.1 Early concepts of heredity

Concept	Proposed	Correct or Incorrect
Pangenesis	Genetic information travels from different parts of the body to reproductive organs.	Incorrect
Inheritance of acquired characteristics	Acquired traits become incorporated into hereditary information.	Incorrect
Preformationism	Miniature organism resides in sex cells, and all traits are inherited from one parent.	Incorrect
Blending inheritance	Genes blend and mix.	Incorrect

Germ-plasm theory	All cells contain a complete set of genetic information.	Correct
Cell theory	All life is composed of cells, and cells arise only from cells.	Correct
Mendelian inheritance	Traits are inherited in accord with defined principles.	Correct

In 1902, after the acceptance of Mendel's theory of heredity, Walter Sutton (1877–1916) proposed that genes, the units of inheritance, are located on chromosomes. Thomas Hunt Morgan (1866–1945) discovered the first mutant fruit fly in 1910 and used fruit flies to unravel many details of transmission genetics. Ronald A. Fisher (1890–1962), John B. S. Haldane (1892–1964), and Sewall Wright (1889–1988) laid the foundation for population genetics in the 1930s by integrating Mendelian genetics and evolutionary theory.

Geneticists began to use bacteria and viruses in the 1940s; the rapid reproduction and simple genetic systems of these organisms allowed detailed study of the organization and structure of their genes. At about this same time, evidence accumulated that DNA was the repository of genetic information. James Watson (b. 1928) and Francis Crick (1916–2004), along with Rosalind Franklin (1920–1958) and Maurice Wilkins (1916–2004), described the three-dimensional structure of DNA in 1953, ushering in the era of molecular genetics (see Chapter 10).

By 1966, the chemical structure of DNA and the system by which it determines the amino acid sequence of proteins had been worked out. Advances in molecular genetics led to the first recombinant

DNA experiments in 1973, which provided techniques for combining genetic material from different sources and touched off another revolution in genetic research. Walter Gilbert (b. 1932) and Frederick Sanger (1918–2013) developed methods for sequencing DNA in 1977. The polymerase chain reaction (PCR), a technique for quickly amplifying tiny amounts of DNA, was developed by Kary Mullis (1944–2019) and others in 1983. PCR has subsequently become one of the most widely used tools in molecular biology.

In 1990, gene therapy was used for the first time to treat human genetic disease in the United States, and the Human Genome Project was launched. By 1995, the first complete DNA sequence of a free-living organism—the bacterium *Haemophilus influenzae*—had been determined, and the first complete sequence of a eukaryotic organism (yeast) was reported a year later. A rough draft of the human genome sequence was reported in 2000 (see Chapter 20), and the sequence was essentially completed in 2003, bringing in another new era in genetics. Building on prior research by several groups, Jennifer Doudna and Emmanuelle Charpentier in 2012 modified the CRISPR-Cas system found in bacteria to develop a powerful new technology for editing the genome. › **TRY PROBLEM 23**

The Cutting Edge of Genetics

With exciting advances being made every year, genetics remains at the forefront of biological research. Rapid methods for sequencing

DNA are being used to sequence the genomes of numerous species, from cockroaches to orangutans to hot peppers to roses. New DNA sequencing techniques are capable of sequencing single DNA molecules, allowing longer and longer sequence reads that facilitate assembling of whole genomes.

Genetics continues to play a major role in agriculture. For example, many people complain about the bland taste of store-bought tomatoes. Geneticists are now using modern genetic techniques to locate genes that are responsible for loss of flavor in commercial tomatoes. In one study, researchers quantified natural compounds that enhance flavor in 400 varieties of tomatoes and identified compounds that were missing or low in poor-tasting commercial varieties. They then used genome sequencing to identify genes that affect the missing flavors, and this information is being used to breed back into commercial varieties compounds that enhance flavor, producing better-tasting tomatoes. Other plant scientists are studying the genetics of volatile organic compounds in flowers, with the goal of engineering fragrance. There is currently a project underway to completely sequence the genomes of 10,000 plant species.

Today, genomic studies provide key insights into evolution. For example, how many types of giraffes are there? Previously, biologists thought there was a single species, but analysis of DNA sequences has now revealed the presence of four distinct species. In 2016, researchers identified the gene responsible for the evolution of

industrial melanism in peppered moths of Britain, where dark moths became more frequent during times of pollution. They discovered that the mutation that produced the dark (*carbonaria*) form of the moths was a large transposable element (a mobile genetic sequence) which inserted into a gene that normally controls cell division and wing development in moths. Whereas archaeologists previously studied fossils, ancient pottery, and tools to understand human ancestry, researchers are now isolating DNA from ancient remains to reconstruct human history. Genome sequences have been analyzed from more than 1000 ancient humans and are being used to answer important questions about the spread of agriculture, the development of languages, and human migration. Genome sequencing of ancient DNA has demonstrated that early *Homo sapiens* interbred with other hominins, including Neanderthals and Denisovans.

Other researchers are unraveling genetic underpinnings of development. Snakes evolved from four-legged ancestors but began to lose their limbs about 150 million years ago. Today, most snakes have lost all vestiges of their limbs (although a few species such as pythons have vestigial pelvises and leg bones). Geneticists have now isolated genes that control the absence of limbs in snakes. When genetic engineers inserted a snake version of an enhancer (a genetic element that controls gene expression) into mice, these mice developed truncated limbs instead of legs.

New research has made CRISPR-Cas9—the powerful genome editing technology that is now widely used in genetic research—more accurate and flexible than ever. New developments have harnessed the ability of CRISPR-Cas9 to provide additional functions, including detection of DNA molecules, editing of RNA, activation of gene expression, and changing of specific base pairs of DNA. CRISPR-Cas was recently used to successfully repair defective genes that cause heart disease in human embryos.

Genetic research continues to provide important information and tools for medicine. Researchers are developing blood tests that detect DNA and proteins shed by tumors, providing the potential for early detection of cancer. Genetics information is being used to develop vaccines to combat infectious diseases such as HIV, Zika, and Ebola. And human gene therapy is being harnessed to treat a number of diseases and disorders, including leukemia, blindness, deafness, blood disorders, cancer, and metabolic disorders. Recently researchers have identified genes that allow Tibetans to live in high altitudes and other genes that allow traditional divers in Southeast Asia to stay underwater for longer periods of time.

As sequencing becomes more affordable, the focus of DNA-sequencing efforts is shifting from the genomes of different species to individual differences within species. In the not-too-distant future, many people will possess a copy of their entire genome sequence, which can be used to help assess the risk of acquiring various diseases and to tailor their treatment should they arise. This

ever-widening scope of genetics raises significant ethical, social, and economic issues.

This brief overview of the history of genetics, from the first domestication of crops to present-day whole-genome sequencing, is not intended to be comprehensive; rather, it is designed to provide a sense of the accelerating pace of advances in genetics. In the chapters to come, we will learn more about the experiments and the scientists who helped shape the discipline of genetics.

THINK-PAIR-SHARE Question 3

CONCEPTS

Humans first applied genetic methods to the domestication of plants and animals between approximately 10,000 and 12,000 years ago. Developments in plant hybridization and cytology in the eighteenth and nineteenth centuries laid the foundation for the field of genetics today. After Mendel's work was rediscovered in 1900, the science of genetics developed rapidly, and today it is one of the most active areas of science.

✔ CONCEPT CHECK 3

How did developments in cytology in the nineteenth century contribute to our modern understanding of genetics?

1.3 A Few Fundamental Concepts Are Important for the Start of Our Journey into Genetics

Undoubtedly, you learned some genetic principles in other biology classes. Let's take a few moments to review some fundamental genetic concepts.

CELLS ARE OF TWO BASIC TYPES: EUKARYOTIC AND PROKARYOTIC

Structurally, cells consist of two basic types, although evolutionarily, the story is more complex (see Chapter 2). Prokaryotic cells lack a nuclear membrane and do not generally possess membrane-bounded organelles, whereas eukaryotic cells are more complex, possessing a nucleus and membrane-bounded organelles such as chloroplasts and mitochondria.

THE GENE IS THE FUNDAMENTAL UNIT OF HEREDITY

The precise way in which a gene is defined often varies depending on the biological context. At the simplest level, we can think of a gene as a unit of information that encodes a genetic characteristic. We

will expand this definition as we learn more about what genes are and how they function.

GENES COME IN MULTIPLE FORMS CALLED ALLELES

A gene that specifies a characteristic may exist in several forms, called alleles. For example, a gene for coat color in cats may exist as an allele that encodes black fur or as an allele that encodes orange fur.

GENES CONFER PHENOTYPES

One of the most important concepts in genetics is the distinction between traits and genes. Traits are not inherited directly. Rather, genes are inherited, and genes, along with environmental factors, determine the expression of traits. The genetic information that an individual organism possesses is its genotype; the trait is its phenotype. For example, the albinism seen in some Hopis is a phenotype, and the information in *OCA2* genes that causes albinism is a genotype.

GENETIC INFORMATION IS CARRIED IN DNA AND RNA

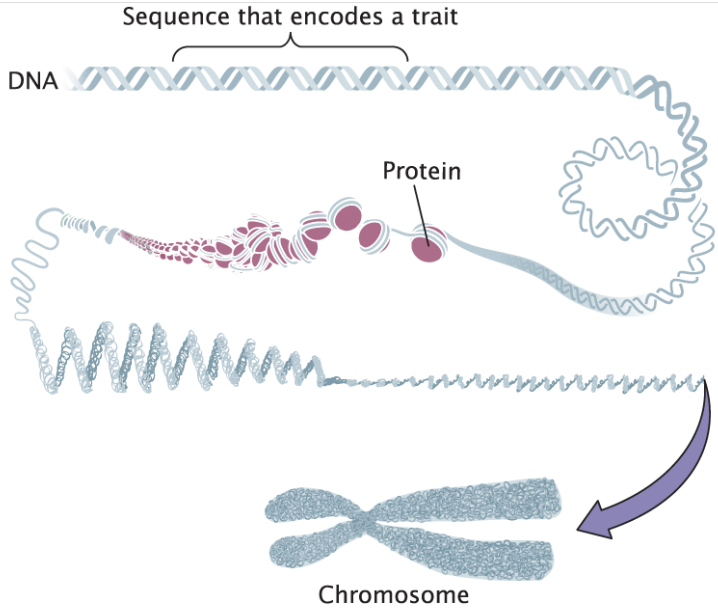
Genetic information is encoded in the molecular structure of nucleic acids, which come in two types: deoxyribonucleic acid (DNA) and

ribonucleic acid (RNA). Nucleic acids are polymers consisting of repeating units called nucleotides; each nucleotide consists of a sugar, a phosphate, and a nitrogenous base. The nitrogenous bases in DNA are of four types: adenine (A), cytosine (C), guanine (G), and thymine (T). The sequence of these bases encodes genetic information. DNA consists of two complementary nucleotide strands. Most organisms carry their genetic information in DNA, but a few viruses carry it in RNA. The four nitrogenous bases of RNA are adenine, cytosine, guanine, and uracil (U).

THINK-PAIR-SHARE Question 4

GENES ARE LOCATED ON CHROMOSOMES

The vehicles of genetic information within a cell are chromosomes (Figure 1.13), which consist of DNA and associated proteins. The cells of each species have a characteristic number of chromosomes; for example, bacterial cells normally possess a single chromosome, human cells possess 46, and pigeon cells possess 80. Genes are located on chromosomes; each chromosome carries a large number of genes.



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020
W. H. Freeman and Company

1.13 Genes are carried on chromosomes, which consist of highly compacted DNA and proteins. Represented here is a typical eukaryotic chromosome.

Description
The illustration shows a long double helix D N A strand. A region on the D N A is labeled “sequence that encodes a trait”. As it proceeds, a turns and forms coils. On further proceeding, spherical proteins are being wrapped up within D N A coils. Then, D N A strand condenses and takes shape of a wire structure. The wire shaped D N A strand continues and finally leads to super-condensed X-shaped chromosome.

CHROMOSOMES SEPARATE THROUGH THE PROCESSES OF MITOSIS AND MEIOSIS

The processes of mitosis and meiosis ensure that a complete set of an organism’s chromosomes exists in each cell that results from cell division. Mitosis is the separation of chromosomes in the division of

somatic (nonsex) cells. Meiosis is the pairing and separation of chromosomes in the division of sex cells to produce gametes (reproductive cells).

GENETIC INFORMATION IS TRANSFERRED FROM DNA TO RNA TO PROTEIN

Many genes encode characteristics by specifying the structure of proteins. Genetic information is first transcribed from DNA into RNA, and then RNA is translated into the amino acid sequence of a protein.

MUTATIONS ARE PERMANENT CHANGES IN GENETIC INFORMATION THAT CAN BE PASSED FROM CELL TO CELL OR FROM PARENT TO OFFSPRING

Gene mutations affect the genetic information of a single gene; chromosome mutations alter the number or the structure of chromosomes and therefore usually affect many genes.

MANY TRAITS ARE AFFECTED BY MULTIPLE FACTORS

Many traits are affected by multiple genes that interact in complex ways with one another and with environmental factors. Human

height, for example, is affected by many genes as well as by environmental factors such as nutrition.

EVOLUTION IS GENETIC CHANGE

Evolution can be viewed as a two-step process: first, genetic variation arises, and second, some genetic variants increase in frequency, whereas other variants decrease in frequency. **› TRY PROBLEM 25**

Chapter 1 Review

CHAPTER SUMMARY

Section 1.1 Learning Objective: Be able to explain the importance of genetics.

- Genetics is central to the life of every person: it influences a person's physical features, personality, intelligence, and susceptibility to numerous diseases.
- Genetics plays important roles in agriculture, the pharmaceutical industry, and medicine. It is central to the study of biology.
- All organisms use similar genetic systems. Genetic variation is the foundation of evolution and is critical to understanding all life.
- A tremendous amount of DNA exists in the biosphere. Scientists use environmental DNA to study and analyze biodiversity.
- The study of genetics can be broadly divided into transmission genetics, molecular genetics, and population genetics.
- Model genetic organisms are species about which much genetic information exists because they have characteristics that make them particularly amenable to genetic analysis.

Section 1.2 Learning Objective: Be able to describe the history of genetics.

- The use of genetics by humans began with the domestication of plants and animals.
- Ancient Greeks developed the concepts of pangenesis and the inheritance of acquired characteristics, both of which were later disproved. Ancient Romans developed practical measures for the breeding of plants and animals.
- Preformationism suggested that a person inherits all of his or her traits from one parent. Blending inheritance proposed that offspring possess a mixture of the parental traits. These ideas were later shown to be incorrect.
- By studying the offspring of crosses between varieties of peas, Gregor Mendel discovered the principles of heredity. Developments in cytology in the nineteenth century led to the understanding that the cell nucleus is the site of heredity.
- In 1900, Mendel's principles of heredity were rediscovered. Population genetics was established in the early 1930s, followed by bacterial and viral genetics. The structure of DNA was discovered in 1953, stimulating the rise of molecular genetics.
- The first human whole-genome sequence was completed in 2003.

Section 1.3 Learning Objective: Be able to explain basic concepts of heredity.

- There are two basic types of cells: prokaryotic and eukaryotic.
- The set of alleles that determines a trait is termed the genotype; the trait that they produce is the phenotype.

- Genes are located on chromosomes, which are made up of nucleic acids and proteins and are partitioned into daughter cells through the process of mitosis or meiosis.
- Genetic information is expressed through the transfer of information from DNA to RNA to proteins.
- Evolution requires genetic change in populations.

IMPORTANT TERMS

genetics
genome
transmission genetics
molecular genetics
population genetics
model genetic organism
pangenesis
inheritance of acquired characteristics
preformationism
blending inheritance
cell theory
germ-plasm theory

ANSWERS TO CONCEPT CHECKS

1. d

2. No, because horses are expensive to house, feed, and propagate; they have too few progeny; and their generation time is too long.
3. Developments in cytology in the 1800s led to the identification of parts of the cell, including the cell nucleus and chromosomes. The cell theory focused biologists' attention on the cell, eventually leading to the conclusion that the nucleus contains the hereditary information.

Chapter 1 Assessment

COMPREHENSION QUESTIONS

Answers to questions and problems preceded by an asterisk can be found at the end of the book.

Section 1.1

1. *How did Hopi culture contribute to the high incidence of albinism among members of the Hopi tribe?
2. Outline some of the ways in which genetics is important to all of us.
3. Give at least three examples of the role of genetics in society today.
4. Briefly explain why genetics is crucial to modern biology.
5. List the three traditional subdisciplines of genetics and summarize what each covers.
6. What are some characteristics of model genetic organisms that make them useful for genetic studies?

Section 1.2

7. *When and where did agriculture first arise?

8. Outline the concept of pangenesis and explain how it differs from the present-day germ-plasm theory.

9. What does the concept of the inheritance of acquired characteristics propose and how is it related to the concept of pangenesis?

10. What is preformationism? What did it have to say about how traits are inherited?

11. Define blending inheritance and contrast it with preformationism.

12. How did developments in botany in the seventeenth and eighteenth centuries contribute to the rise of modern genetics?

13. List some advances in genetics made in the twentieth century.

14. Briefly explain the contribution that each of the following people made to the study of genetics.

    a. Matthias Schleiden and Theodor Schwann

    b. August Weismann

    c. Gregor Mendel

d. James Watson, Francis Crick, Rosalind Franklin, and Maurice Wilkins

e. Kary Mullis

Section 1.3

15. *What are the two basic cell types (from a structural perspective) and how do they differ?

16. Summarize the relations between genes, DNA, and chromosomes.

APPLICATION QUESTIONS AND PROBLEMS

Section 1.1

17. *How are genetics and evolution related?

18. *For each of the following genetic topics, indicate whether it focuses on transmission genetics, molecular genetics, or population genetics.

    a. Analysis of pedigrees to determine the probability of someone inheriting a trait

b. Study of people on a small island to determine why a genetic form of asthma is prevalent on the island

c. Effect of nonrandom mating on the distribution of genotypes among a group of animals

d. Examination of the nucleotide sequences found at the ends of chromosomes

e. Mechanisms that ensure a high degree of accuracy in DNA replication

f. Study of how the inheritance of traits encoded by genes on sex chromosomes (sex-linked traits) differs from the inheritance of traits encoded by genes on nonsex chromosomes (autosomal traits)

19. How does the picture in **Figure 1.6a** illustrate transmission genetics?

20. Describe some of the ways in which your own genetic makeup affects you as a person. Be as specific as you can.

21. Describe at least one trait that appears to run in your family (appears in multiple members of the family). Does this trait run in your family because it is an inherited trait or because it is caused by environmental factors that are common to family members? How might you distinguish between these possibilities?

Section 1.2

22. *Genetics is said to be both a very old science and a very young science. Explain what is meant by this statement.

23. *Match each of the descriptions (*a* through *d*) with the appropriate theory or concept that follows.

    Preformationism

    Pangenesis

    Germ-plasm theory

    Inheritance of acquired characteristics

    a. Each reproductive cell contains a complete set of genetic information.

    b. All traits are inherited from one parent.

    c. Genetic information may be altered by the use of a characteristic.

    d. Cells of different tissues contain different genetic information.

24. Briefly explain why each of the following theories is incorrect:

    a. Pangenesis

    b. Preformationism

c. Blending inheritance

d. Inheritance of acquired characteristics

Section 1.3

25. *Compare and contrast the following terms:

a. Eukaryotic and prokaryotic cells

b. Gene and allele

c. Genotype and phenotype

d. DNA and RNA

e. DNA and chromosome

CHALLENGE QUESTIONS

Introduction

26. *The type of albinism that arises with high frequency among Hopi Native Americans (discussed in the introduction to this chapter) is most likely oculocutaneous albinism type 2, due to a defect in the *OCA2* gene on chromosome 15. Visit the Online Mendelian Inheritance in Man Website (http://www.ncbi.nlm.nih.gov/omim/) and search the database for albinism. Based on this information, how does the

phenotype of oculocutaneous albinism type 2 differ from the phenotypes of other forms of albinism in humans? What genes are mutated in other types of albinism?

Section 1.1

27. We now know a great deal about the genetics of humans, and humans are the focus of many genetic studies. What are some of the reasons humans have been the focus of intensive genetic study?

28. Use Google Scholar (https://scholar.google.com/) or another appropriate search engine to find research articles published in the past five years that focus on (a) transmission genetics, (b) molecular genetics, and (c) population genetics. Give an example of each.

Section 1.3

29. *Suppose that life exists elsewhere in the universe. All life must contain some type of genetic information, but alien genomes might not consist of nucleic acids and have the same features as those found in the genomes of life on Earth. What might be the common features of all genomes, no matter where they exist?

30. Choose one of the ethical or social issues in parts *a* through *e* and give your opinion on the issue. For background information, you might read one of the articles on ethics

marked with an asterisk in the Suggested Readings section for Chapter 1 in your Sapling Plus.

a. Should a person's genetic makeup be used in determining his or her eligibility for life insurance?

b. Should biotechnology companies be able to patent newly sequenced genes?

c. Should gene therapy be used in people?

d. Should genetic testing be made available for inherited disorders for which there is no treatment or cure?

31. A 45-year-old woman undergoes genetic testing and discovers that she is at high risk for developing colon cancer and Alzheimer disease. Because her children have 50% of her genes, they may also be at an increased risk for these diseases. Does she have a moral or legal obligation to tell her children and other close relatives about the results of her genetic testing?

32. Suppose that you could undergo genetic testing at age 18 for susceptibility to a genetic disease that would not appear until middle age and has no available treatment.

    a. What would be some of the possible reasons for having such a genetic test and some of the possible reasons for not having the test?

    b. Would you personally want to be tested? Explain your reasoning.

💬 ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Think-Pair-Share questions are designed to be worked in collaboration with other students. First THINK about the question, then PAIR up with one or more other students, and finally SHARE your answers and work together to arrive at a solution.

Section 1.1

1. Bob says that he is healthy and has no genetic diseases such as hemophilia or Down syndrome. Therefore, he says, genetics plays little role in his life. Do you think Bob is correct in his conclusion? Why or why not?

2. Are mutations good or bad? Explain your answer.

Section 1.2

3. Propose some arguments for and against the development of genetically engineered foods (genetically modified organisms, or GMOs)? Think about the economic and environmental benefits, health risks, ecological effects, and social impact of their use. List some reasons for and against genetically engineering the foods we eat and the types of evidence that would be needed to support these arguments.

Section 1.3

4. Why do you think all organisms use nucleic acids for encoding genetic information? Why not use proteins or carbohydrates? What advantages might DNA have as the source of genetic information?

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

A concept map, often organized around a question, is designed to help you think about the relationships between concepts. First think of concepts and terms that address the question and the relationships among them. Then create a diagram to show these relationships. In the diagram, draw a circle or box around each concept or term. Represent the relationships between concepts with lines that connect the concepts. Each line should have an arrow, showing the direction of the relationship. For each connecting line, add a label consisting of a few words that explains the relationship between the concepts. Include as many connections as you can.

It is important to recognize that there is not one, single correct map for a set of concepts; the same concepts may be organized in different ways.

Introduction

1. Explain the relationship between genetics and albinism in the Hopi Native Americans by creating a concept map that links the following terms and phrases: albinism, reduced melanin, autosomal recessive, oculocutaneous albinism type II, *OCA2* gene, Hopi Native Americans, mating advantage, small size of the tribe, reduced selection against, light skin, sunburn, skin cancer, visual impairment.

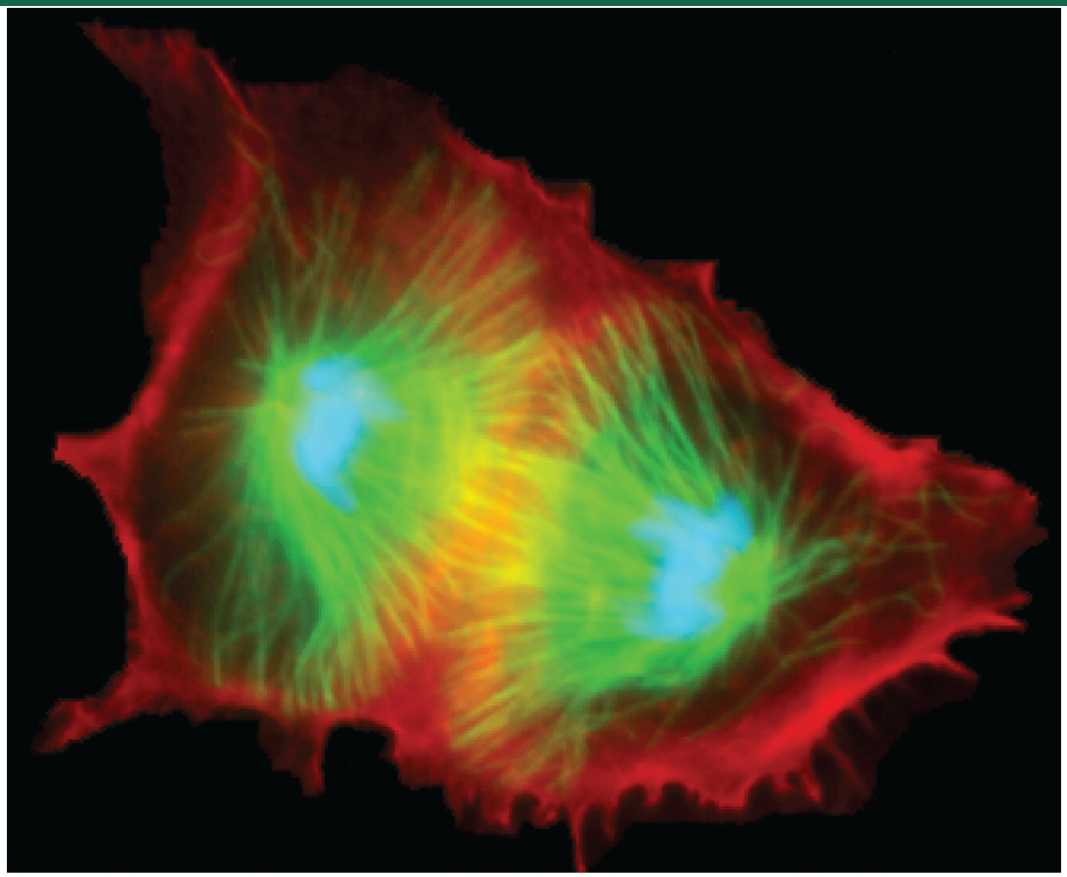
Section 1.3

2. Outline the organization of genetic information within a cell by creating a concept map that links the following terms: DNA, genes, alleles, genotype, phenotype, chromosomes, proteins, environmental factors, genome.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 2

Chromosomes and Cellular Reproduction



Courtesy of Julie Canman and Ted Salman.

A rat kangaroo kidney cell undergoing mitosis, the process through which each new cell receives a complete copy of the genetic material. Chromosomes are shown in blue.

The Blind Men's Riddle

In a well-known riddle, two blind men by chance enter a department store at the same time, go to the same counter, and both order five pairs of socks, each pair a different color. The salesclerk is so befuddled by this strange coincidence that he places all ten pairs (two black pairs, two blue pairs, two gray pairs, two brown pairs, and two green pairs) into a single shopping bag, gives the bag of socks to one blind man, and gives an empty bag to the other. The two blind men happen to meet on the street outside, where they discover that one of their bags contains all ten pairs of socks. How do the blind men, without seeing and without any outside help, sort out the socks so that each man goes home with exactly five pairs of different colored socks? Can you come up with a solution to the riddle?

By an interesting coincidence, cells face the same challenge as that of the blind men. Most organisms possess two sets of genetic information, one set inherited from each parent. Before cell division, the DNA in each chromosome replicates; after replication, there are two copies—called sister chromatids—of each chromosome. At the end of cell division, it is critical that each of the two new cells receives a complete copy of the genetic material, just as each blind man needs to go home with a complete set of socks.

The solution to the riddle is simple. Socks are sold as pairs; the two socks of a pair are typically connected by a thread. As a pair is removed from the bag, the men each grasp a different sock of the

pair and pull in opposite directions. When the socks are pulled tight, one of the men can take a pocket knife and cut the thread connecting the pair. Each man then deposits his single sock in his own bag. At the end of the process, each man's bag will contain exactly two black socks, two blue socks, two gray socks, two brown socks, and two green socks.[1]

Remarkably, cells employ a similar solution for separating their chromosomes into new daughter cells. As we learn in this chapter, the replicated chromosomes line up at the center of a cell undergoing division, and like the socks in the riddle, the sister chromatids of each chromosome are pulled in opposite directions. Like the thread connecting two socks of a pair, a molecule called cohesin holds the sister chromatids together until it is severed by a molecular knife called separase. The two resulting chromosomes separate, and the cell divides. This process ensures that a complete set of chromosomes is deposited in each cell.

In this analogy, the blind men and cells differ in one critical regard: if the blind men make a mistake, one man ends up with an extra sock and the other is a sock short, but no great harm results. The same cannot be said for human cells. Errors in chromosome separation, producing cells with too many or too few chromosomes, are frequently catastrophic, leading to cancer, miscarriage, or—in some cases—a child with severe disabilities.

THINK-PAIR-SHARE

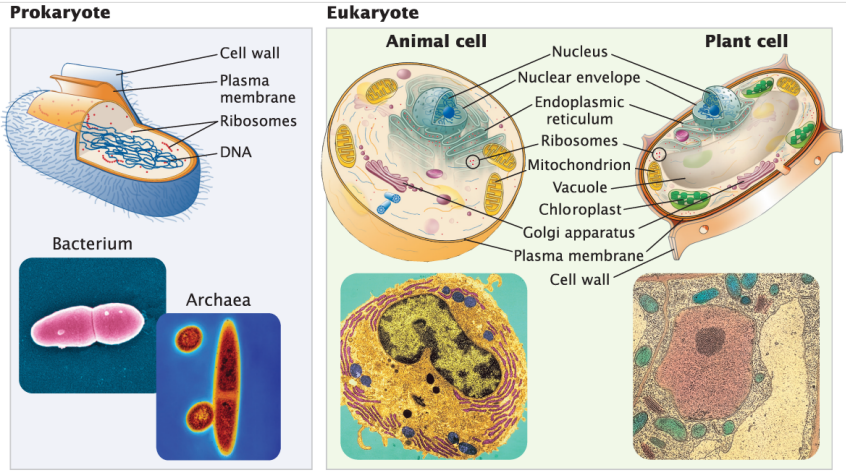
- In the blind men's riddle, two blind men must sort out ten pairs of socks so that each man gets exactly five pairs of different colored socks. In the analogy, is it important that the men are blind? In a cell, what does the blindness represent?

This chapter explores the process of cell reproduction and explains how a complete set of genetic information is transmitted to new cells. In prokaryotic cells, reproduction is relatively simple because prokaryotic cells tend to possess only one chromosome per cell. In eukaryotic cells, multiple chromosomes must be copied and distributed to each of the new cells, making cell reproduction more complex. Cell division in eukaryotes takes place through mitosis or meiosis, processes that serve as the foundation for much of eukaryotic genetics.

Grasping the processes of mitosis and meiosis requires more than simply memorizing the sequences of events that take place in each stage, although these events are important. The key is to understand how genetic information is apportioned in the course of cell reproduction through the dynamic interplay of DNA synthesis, chromosome movement, and cell division. These processes also underlie the transmission of genetic information from parent to progeny and are the basis of the similarities and differences between parents and their offspring.

2.1 Prokaryotic and Eukaryotic Cells Differ in a Number of Genetic Characteristics

Biologists have traditionally classified all living organisms into two major groups, the prokaryotes and the eukaryotes (Figure 2.1). A **prokaryote** is a unicellular organism with a relatively simple cell structure. A **eukaryote** has a compartmentalized cell structure with components bounded by intracellular membranes; eukaryotes may be either unicellular or multicellular.



	Prokaryotic cells	Eukaryotic cells
Nucleus	Absent	Present
Cell diameter	Relatively small, from 1 to 10 μm	Relatively large, from 10 to 100 μm
Genome	Usually one circular DNA molecule	Multiple linear DNA molecules
DNA	Not complexed with histones in bacteria; some histones in archaea	Complexed with histones
Amount of DNA	Relatively small	Relatively large
Membrane-bounded organelles	Absent	Present

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Photographs (left to right): Dr. Gary D. Gaugler/Newscom; Dr. Kari Lounatmaa/Science Source; Dr. Gopal Murti/ Medicalimages.com; Biophoto Associates/Science Source.

2.1 Prokaryotic and eukaryotic cells differ in structure.

Description

The illustration on the left shows a diagram of a prokaryotic cell and two micrographs. The illustration on the right shows a diagram of a eukaryotic animal cell and plant cell and two micrographs. A table present below compares the differences between prokaryotic and eukaryotic cells.

In the first illustration, a diagram shows cross-section of a rod-shaped prokaryotic cell. The cell has an outer cell wall with various thread-like structures and the plasma membrane underneath. Tiny spheres inside the cell are labeled as ribosomes and an intertwined stranded material is labeled as D N A. Two micrographs show a bacterium and Archaea which are both rod-shaped.

In the second illustration, a diagram shows components of a eukaryotic animal and plant cell. The nucleus, a spherical structure with several wavy layers outside, is located in center of both the cells. The folded layers extending out from the nuclei are components of the endoplasmic reticulum. The inside of the nucleus contains a sphere and D N A. The nucleus is enclosed in a nuclear envelope. Both cell diagrams also consist of ribosomes, mitochondria, Golgi apparatus, and a plasma membrane. The ribosomes are represented by tiny spheres. Mitochondria, oblong enveloped structures with the internal portion of the envelope membrane extending inwards into a number of stacked folds, appears in both cells. The Golgi apparatus, thin stacked cluster of ovular-shaped layers, is present in both the cells. Organelles that are only present in the plant cell are the vacuole, chloroplasts, and cell wall. The vacuole is large and is ovular-shaped which occupies about 50 percent of the inside of the plant cell. The chloroplasts, green-colored ovular membrane-bound objects with multiple spherical structures inside, are only present in the plant cell. Two micrographs below show a eukaryotic animal and plant cell, respectively.

A table below highlights the structural differences between a eukaryotic and prokaryotic cell. The table has 6 rows and 3 columns. The information present in the table is as follows:

Nucleus: Prokaryotic cells, Absent. Eukaryotic cells, present.

Cell diameter: Prokaryotic cells, relatively small from 1 to 10 micrometers. Eukaryotic cells, relatively large from 10 to 100 micrometers.

Genome: Prokaryotic cells, usually one circular D N A molecule. Eukaryotic cells, multiple linear D N A molecules.

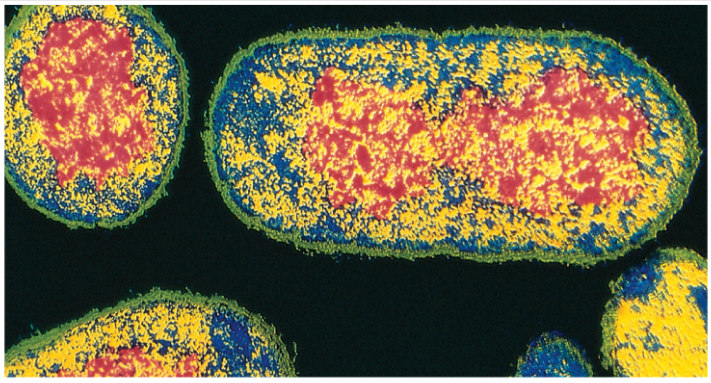
D N A: Prokaryotic cells, not complexed with histones in bacteria; some histones in archaea. Eukaryotic cells, complexed with histones.

Amount of D N A: Prokaryotic cells, relatively small. Eukaryotic cells, relatively large.

Membrane-bounded organelles: Prokaryotic cells, absent. Eukaryotic cells, present.

Research indicates, however, that the division of life is not so simple. Although all prokaryotes are similar in cell structure, they include at least two fundamentally distinct types: the **bacteria** (also called eubacteria or "true bacteria") and the **archaea** ("ancient bacteria"). An examination of equivalent DNA sequences reveals that bacteria and archaea are as distantly related to each other as they are to the eukaryotes. Thus, from an evolutionary perspective, there are three major groups of organisms: bacteria, archaea, and eukaryotes. Although bacteria and archaea are similar in cell structure, some genetic processes in archaea (such as transcription) are more similar to those in eukaryotes, and the archaea may be closer evolutionarily to eukaryotes than to bacteria. Some researchers propose that eukaryotes arose within the archaea and thus that there are two primary domains of life: the bacteria and another domain that includes the archaea and the eukaryotes. The evolutionary relationships among bacteria, archaea, and eukaryotes remain uncertain, however, and are the focus of current research. In this book, the prokaryotic–eukaryotic distinction will be made frequently, but important bacterial–archaeal differences will also be noted.

From the perspective of genetics, a major difference between prokaryotic and eukaryotic cells is that a eukaryotic cell has a nuclear membrane, which surrounds the genetic material to form a **nucleus** and separates the DNA from the other cellular contents. In prokaryotic cells, the genetic material is in close contact with other components of the cell—a property that has important consequences for the way in which gene expression is controlled (**Figure 2.2**).



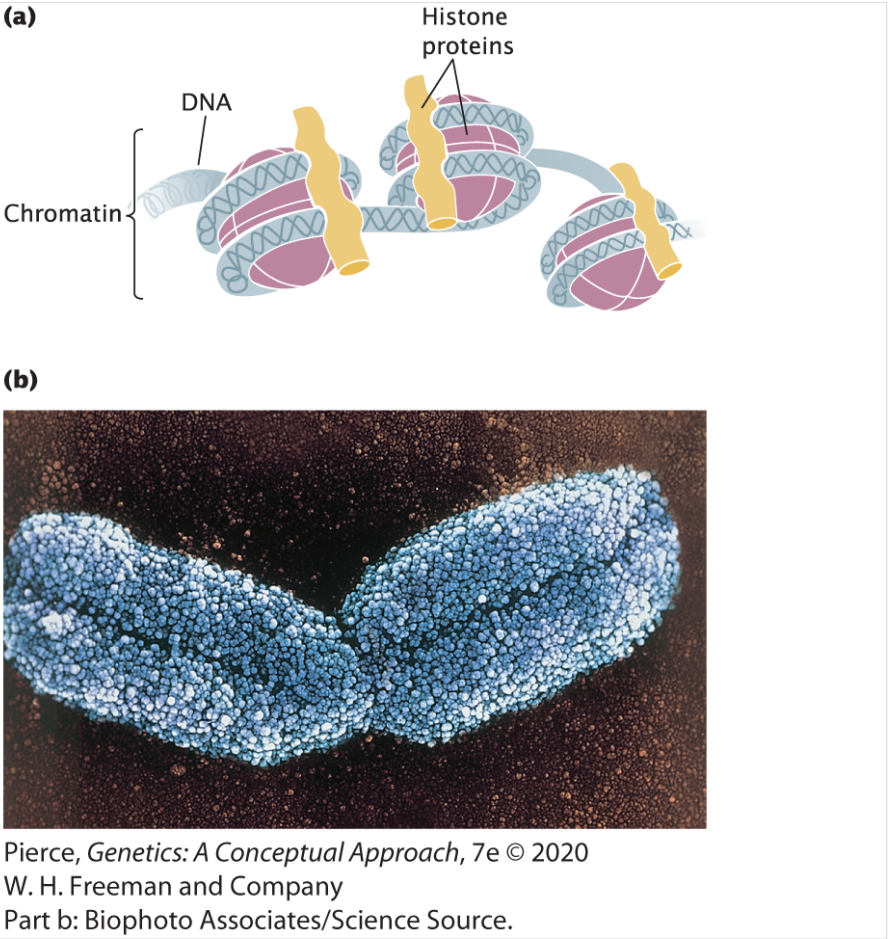
A. Barry Dowsett/Science Source.

2.2 Prokaryotic DNA (shown in red) is neither surrounded by a nuclear membrane nor complexed with histone proteins.

Description
The prokaryotic D N A is not surrounded by a nuclear membrane and is also not complexed with histone proteins.

Another fundamental difference between prokaryotes and eukaryotes lies in the packaging of their DNA. In eukaryotes, DNA associates closely with a special class of proteins, the **histones**, to form tightly packed chromosomes (**Figure 2.3**). This complex of

DNA and histone proteins, called **chromatin**, is the stuff of eukaryotic chromosomes. Histone proteins help regulate the accessibility of DNA to enzymes and other proteins that copy and read the DNA (see Chapter 17), and they also enable the DNA to fit into the nucleus of a eukaryotic cell (Chapter 11). Archaea also have some histone proteins that complex with DNA. The histones found in archaea are related to but distinct from those found in eukaryotes, and the structure of archaeal chromatin is different from that found in eukaryotes. Bacteria do not possess histones, and their DNA does not exist in the highly ordered, tightly packed arrangement found in eukaryotic cells. The copying and reading of DNA are therefore simpler processes in bacteria.



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2.3 Eukaryotic chromosomes consist of DNA and histone proteins. (a) DNA wraps around the histone proteins to form chromatin, the material that makes up chromosomes. (b) A eukaryotic chromosome.

Description

Illustration “a” at the top shows a chromatin formed by a D N A duplex wrapped around clusters of histone proteins. The D N A duplex strand wraps twice around each nucleosome. Another histone protein, thin and cylindrical shaped, is attached outside each of the condensed balls formed by D N A wrapped histones.

The micrograph “b” at the bottom shows a eukaryotic chromosome which is X-shaped. It has two lobes on each side with tiny spherical bodies on its surface.

The genes of prokaryotic cells are generally located on a single circular molecule of double-stranded DNA—the chromosome of a prokaryotic cell. In eukaryotic cells, genes are located on multiple, usually linear DNA molecules (multiple chromosomes). Eukaryotic cells therefore require mechanisms to ensure that a copy of each chromosome is faithfully transmitted to each new cell. However, this generalization—a single circular chromosome in prokaryotes and multiple linear chromosomes in eukaryotes—is not always true. A few bacteria have more than one chromosome, and important bacterial and archaeal genes are frequently found on other DNA molecules called *plasmids* (see Chapter 9). Furthermore, in some eukaryotes, a few genes are located on circular DNA molecules found in certain organelles, such as mitochondria and chloroplasts (see Chapter 11).

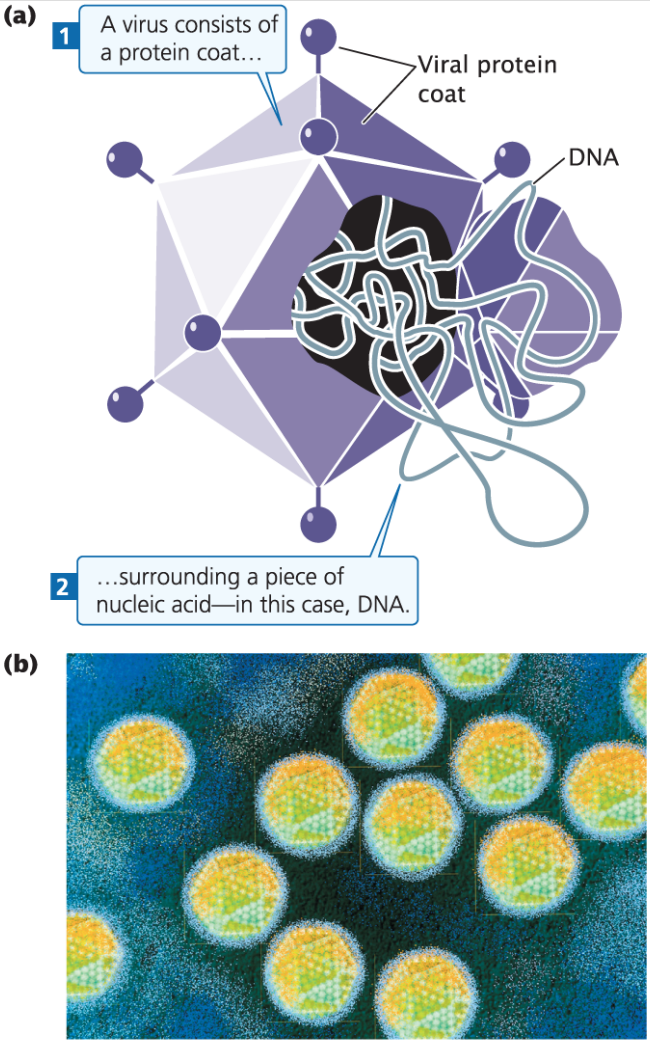
CONCEPTS

Organisms are classified as prokaryotes or eukaryotes, and the prokaryotes consist of archaea and bacteria. A prokaryote is a unicellular organism that lacks a nucleus, and its genome is usually a single chromosome. Eukaryotes may be either unicellular or multicellular, their cells possess a nucleus, their DNA is complexed with histone proteins, and their genomes consist of multiple chromosomes.

✔ CONCEPT CHECK 1

List several characteristics that bacteria and archaea have in common and that distinguish them from eukaryotes.

Viruses are neither prokaryotic nor eukaryotic because they do not possess the structure of a cell. Viruses are actually simple structures composed of an outer protein coat surrounding nucleic acid (either DNA or RNA; **Figure 2.4**). All known viruses can reproduce only within host cells, and their evolutionary relationship to cellular organisms is uncertain. Their simplicity and small genomes make viruses useful for studying some molecular processes and for some types of genetic analyses. For example, bacteriophages—viruses that infect bacteria—have been used to study the control of gene expression, recombination, and molecular evolution.



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2.4 A virus is a simple replicative structure consisting of protein and nucleic acid. (a) Structure of an adenovirus. (b) A micrograph of adenoviruses.

Description

The diagram “a” shows a purple colored icosahedral-shaped virus, the outside layer is labeled as the protein coat, which is surrounding the virus’s D N A. Each edge of the protein coat has a protruding thin rod with a round structure at its end. The viral D N A is like a long thin grey thread inside the coat. A caption reads, “A virus consists of a protein coat surrounding a piece of nucleic acid—in this case, D N A.” The micrograph “b” shows a number of circular greenish yellow colored viruses over a blue colored background.

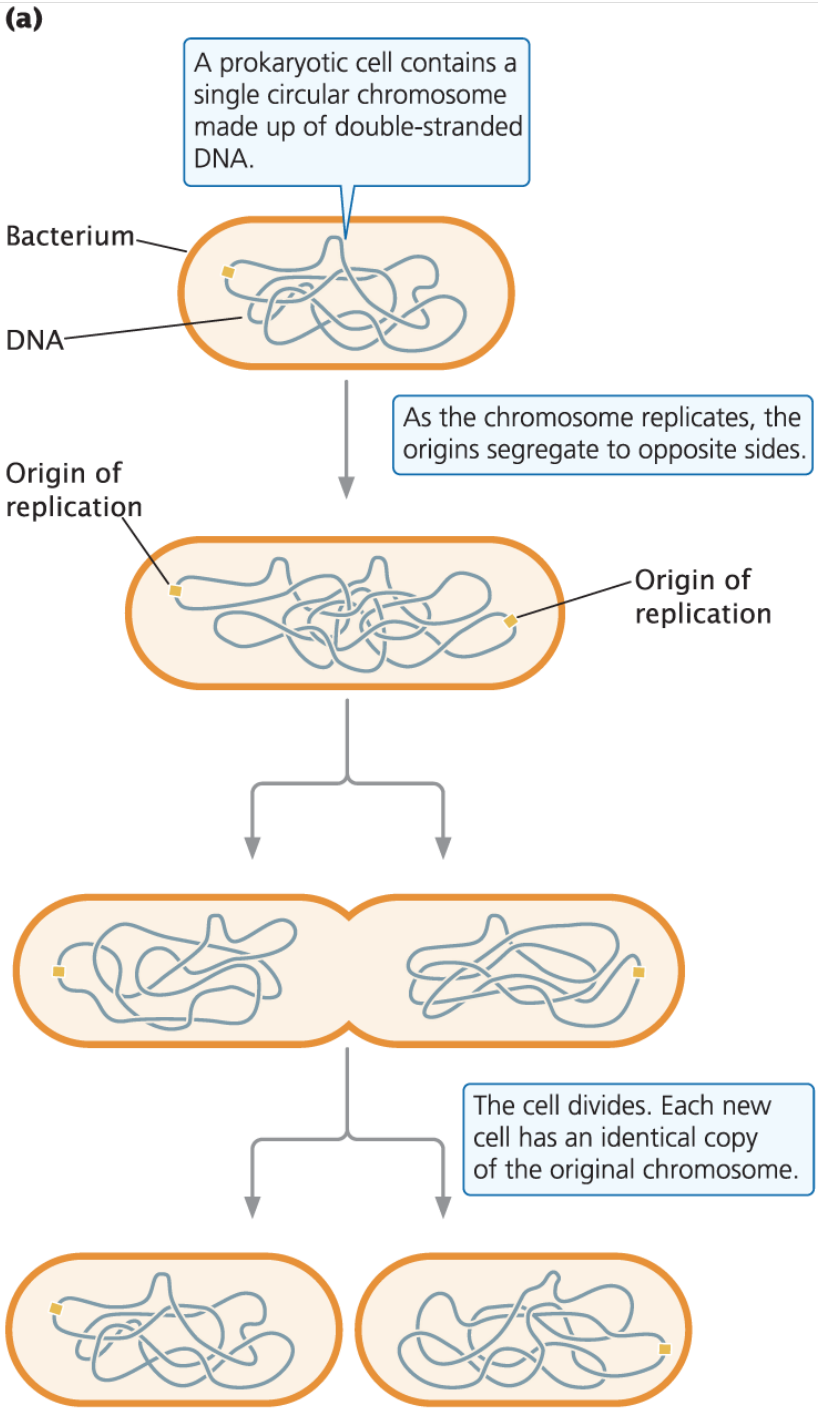
2.2 Cell Reproduction Requires the Copying of the Genetic Material, Separation of the Copies, and Cell Division

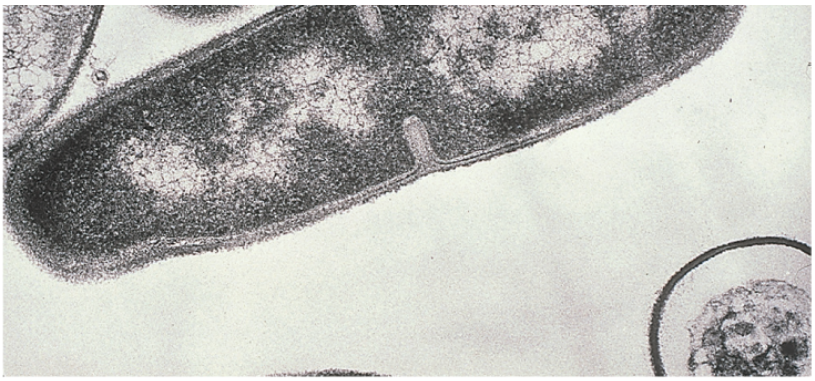
For any cell to reproduce successfully, three fundamental events must take place: (1) its genetic information must be copied, (2) the copies must be separated from each other, and (3) the cell must divide. All cellular reproduction includes these three events, but the processes that lead to these events differ in prokaryotic and eukaryotic cells because of their structural differences.

Prokaryotic Cell Reproduction by Binary Fission

When a prokaryotic cell reproduces, its circular chromosome replicates, and the cell divides in a process called *binary fission* (Figure 2.5). Replication usually begins at a specific place on the circular chromosome, called the origin of replication. The origins of the two newly replicated chromosomes move away from each other and toward opposite ends of the cell. Proteins called structural maintenance of chromosomes (SMC) complexes encircle the DNA and help keep the two newly synthesized chromosomes from getting

tangled as they are replicated. Finally, a new cell wall forms between the two chromosomes, producing two cells, each with an identical copy of the chromosome. Under optimal conditions, some bacterial cells divide every 20 minutes. At this rate, a single bacterial cell could produce a billion descendants in a mere 10 hours.


(a)
A prokaryotic cell contains a single circular chromosome made up of double-stranded DNA.
Bacterium
DNA
As the chromosome replicates, the origins segregate to opposite sides.
Origin of replication
Origin of replication
The cell divides. Each new cell has an identical copy of the original chromosome.
(b)



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Part b: Lee D. Simon/Science Source.

2.5 Prokaryotic cells reproduce by binary fission. (a) The process of binary fission. (b) A bacterial cell undergoing binary fission.

Description

The illustration “a” at the top shows a bacterium with a circular D N A double strand. The illustration shows the steps from top to bottom. A caption reads, prokaryotic cell contains a single circular chromosome made up of double-stranded D N A with a yellow-colored spherical origin of replication. This stage progresses to the next stage where the chromosome replicates. A caption reads, as the chromosome replicates, the origins segregate to opposite sides. The resultant cell has replicated D N A where the origins of replication are anchored to opposite sides of the cell. This progresses to the next stage where two cells are distinct with a furrow between them. Each cell shows one circular D N A double strand. A caption reads, the cell divides. Each new cell has an identical copy of the original chromosome. This leads to formation of two daughter cells.

The micrograph “b” at the bottom shows a bacterial cell undergoing binary fission with a furrow forming at its center.

Eukaryotic Cell Reproduction

Like prokaryotic cell reproduction, eukaryotic cell reproduction requires the processes of DNA replication, copy separation, and division of the cytoplasm. However, the presence of multiple DNA molecules requires a more complex mechanism to ensure that exactly one copy of each DNA molecule ends up in each of the new cells.

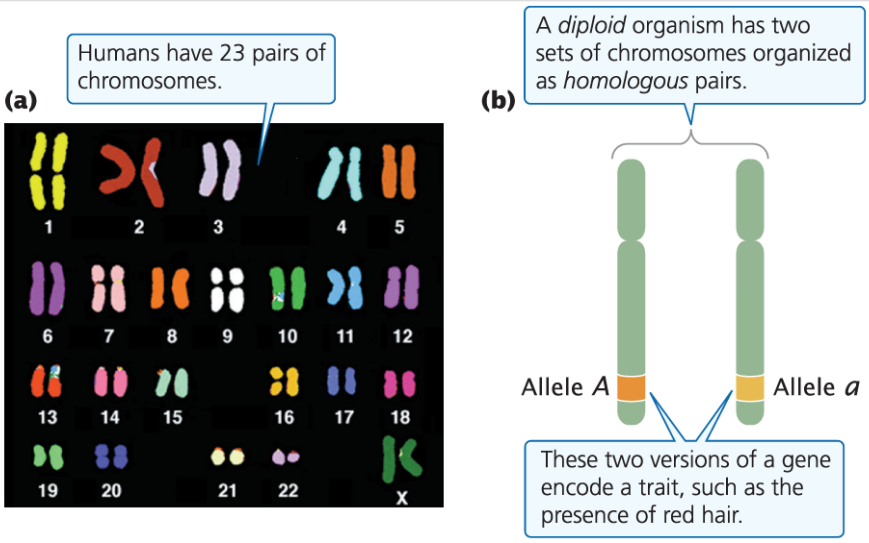
Eukaryotic chromosomes are separated from the cytoplasm by the nuclear membrane. The nucleus has a highly organized internal scaffolding, called the nuclear matrix, which consists of a network of protein fibers. The nuclear matrix maintains precise spatial relations among the components of the nucleus and takes part in DNA replication, the expression of genes, and the modification of gene products before they leave the nucleus.

EUKARYOTIC CHROMOSOMES

Each eukaryotic species has a characteristic number of chromosomes per cell: potatoes have 48 chromosomes, fruit flies have 8, and humans have 46. There appears to be no special relation between the complexity of an organism and its number of chromosomes per cell.

In most eukaryotic cells, there are two sets of chromosomes. The presence of two sets is a consequence of sexual reproduction: one set is inherited from the male parent and the other, from the female parent. Each chromosome in one set has a corresponding

chromosome in the other set; together, the two chromosomes constitute a **homologous pair** (Figure 2.6). Human cells, for example, have 46 chromosomes: in females, there are 23 homologous pairs; in males, 22 pairs are homologous, and the last pair (X and Y chromosomes) is only partially homologous (see Chapter 4).



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Part a: Courtesy of Dr. Thomas Ried and Dr. Evelin Schrock.

2.6 Diploid eukaryotic cells have two sets of chromosomes. (a) A set of chromosomes from a female human cell. Each pair of chromosomes has been hybridized to a uniquely colored probe, giving it a distinct color. (b) The chromosomes are present in homologous pairs. Each pair consists of two chromosomes that are alike in size and structure and carry information for the same characteristics.

Description
The Karyotype “a” on the left shows 23 sets of human chromosomes that vary in color, shape and size. The chromosomes are not attached into an X shaped formation, but are present as separate entities. The twenty third pair is labeled as X. A caption reads, humans have 23 pairs of chromosomes.

The illustration “b” on the right shows two non-conjoined chromosomes. A caption reads, a diploid organism has two sets of chromosomes organized as homologous pairs. The chromosome on the left has a band at the bottom chromatid, labeled allele big A. The chromosome on the right has a band at the bottom chromatid labeled as allele small a. A caption reads, these two versions of a gene encode a trait, such as the presence of red hair.

The two chromosomes of a homologous pair are usually alike in structure and size, and each carries genetic information for the same set of hereditary characteristics (the sex chromosomes are an exception and will be discussed in Chapter 4). For example, if a gene on a particular chromosome encodes a characteristic such as hair color, another copy of the gene (each copy is called an *allele*) at the same position on that chromosome’s homolog *also* encodes hair color. However, these two alleles need not be identical: one might encode brown hair and the other might encode blond hair.

Cells that carry two sets of genetic information are **diploid**. In general, the ploidy of the cell indicates how many sets of genetic information it possesses. The reproductive cells of eukaryotes (such as eggs, sperm, and spores), and even the nonreproductive cells of some eukaryotic organisms, contain a single set of chromosomes and are **haploid**. The cells of some other eukaryotes contain more than two sets of genetic information and are therefore called **polyploid** (see Chapter 8).

CONCEPTS

Cells reproduce by copying their genetic information, separating the copies, and then dividing. Because eukaryotic cells possess multiple chromosomes, mechanisms exist to ensure that each new cell receives one copy of each chromosome. Most eukaryotic cells are diploid, and their two chromosome sets can be arranged in homologous pairs. Haploid cells contain a single set of chromosomes.

✔ CONCEPT CHECK 2

Diploid cells have

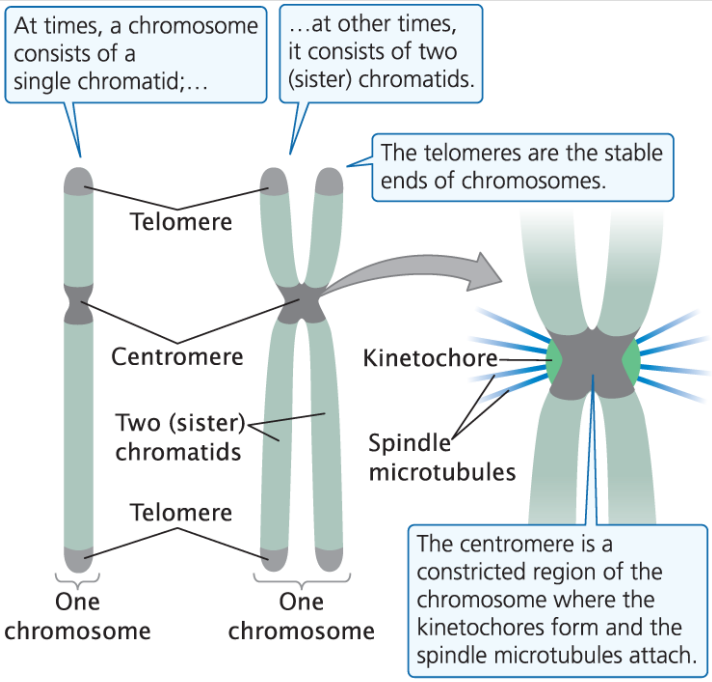
a. two chromosomes.
b. two sets of chromosomes.
c. one set of chromosomes.
d. two pairs of homologous chromosomes.

CHROMOSOME STRUCTURE

The chromosomes of eukaryotic cells are larger and more complex than those found in prokaryotes, but each unreplicated chromosome nevertheless consists of a single molecule of DNA. Although linear, the DNA molecules in eukaryotic chromosomes are highly folded and condensed; if stretched out, some human chromosomes would be several centimeters long—thousands of times as long as the span of a typical nucleus. To package such a tremendous length of DNA into the small volume of the nucleus, each DNA molecule is coiled around histone proteins and tightly packed, forming a rod-shaped chromosome. Most of the time, the chromosomes are thin and difficult to observe, but before cell

division, they condense further into thick, readily observed structures; it is at this stage that chromosomes are usually studied.

A functional chromosome has three essential elements: a centromere, a pair of telomeres, and origins of replication. The **centromere** appears as a constricted region on the chromosome (**Figure 2.7**). It serves as the attachment point for spindle microtubules—the filaments responsible for moving chromosomes in cell division. Before cell division, a multiprotein complex called the kinetochore assembles on the centromere; later, spindle microtubules attach to the kinetochore (see Figure 2.7). Chromosomes lacking a centromere cannot be drawn into the newly formed nuclei; such chromosomes are lost, often with catastrophic consequences for the cell. On the basis of the location of the centromere, chromosomes are classified into four types: metacentric, submetacentric, acrocentric, and telocentric (**Figure 2.8**).



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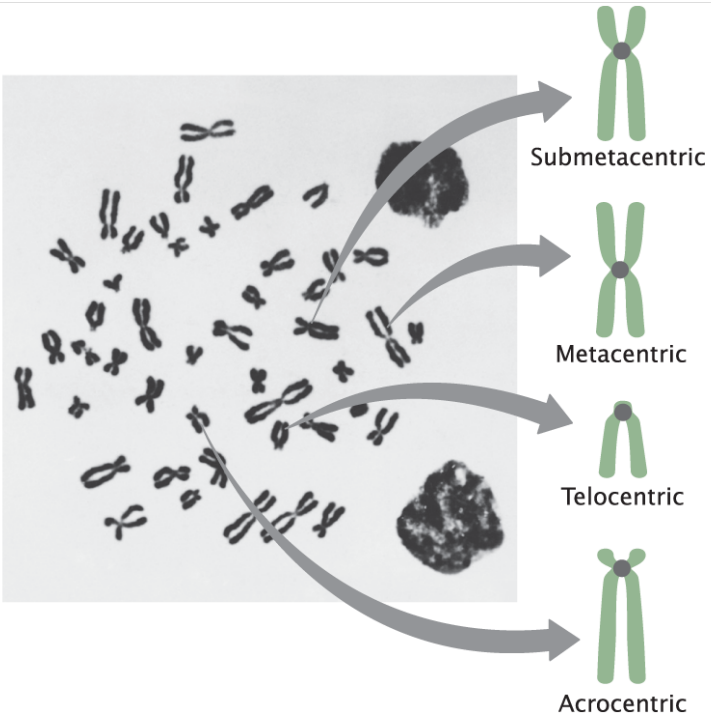
2.7 Each eukaryotic chromosome has a centromere and telomeres.

Description

The diagram on the left shows a single rod-shaped chromatid and the diagram in the middle shows 2 sister chromatids conjoined near the middle at the centromere thereby forming an X-shaped chromosome. At the ends of short arms of the chromatids in both diagrams are the telomeres, or the stable ends of the chromosomes. Three captions read, at times, a chromosome consists of a single chromatid at other times, it consists of two (sister) chromatids. The telomeres are the stable ends of chromosomes. The diagram on the right is an up-close picture of the centromere. The kinetochore appears in the diagram as a structure overlaying the entire outside of the centromere, and the spindle microtubules are straight lines touching the kinetochore and extending outward. A caption reads, the centromere is a constricted region of the chromosome where the kinetochores form and the spindle microtubules attach.



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2.8 Eukaryotic chromosomes can be divided into four major types based on the position of the centromere.

Description

A magnified view of each type of condensed X-shaped chromosomes is presented in the right side. In a submetacentric chromosome, the centromere is located a little above the center. In a metacentric chromosome, the centromere is located at the center. In a telocentric chromosome, the centromere is located at the terminal end of the chromosome. In an acrocentric chromosome, the centromere is located almost near one end of the chromosome.

Telomeres are the specific DNA sequences and associated proteins located at the tips of whole linear chromosomes (see Figure 2.7). Just as plastic tips protect the ends of a shoelace, telomeres protect and stabilize the chromosome ends. If a chromosome breaks, producing

new ends, the chromosome is degraded at the newly broken ends. Telomeres provide chromosome stability. Research shows that telomeres also participate in limiting cell division and may play important roles in aging and cancer (discussed in Chapter 12).

Origins of replication are the sites where DNA synthesis begins; unlike centromeres and telomeres, they are not easily observed by microscopy. Their structure and function will be discussed in more detail in Chapter 12.

In preparation for cell division, each chromosome replicates, making a copy of itself, as already mentioned. These two initially identical copies, called **sister chromatids**, are held together at the centromere (see Figure 2.7). Each sister chromatid consists of a single molecule of DNA.

THINK-PAIR-SHARE Question 1

CONCEPTS

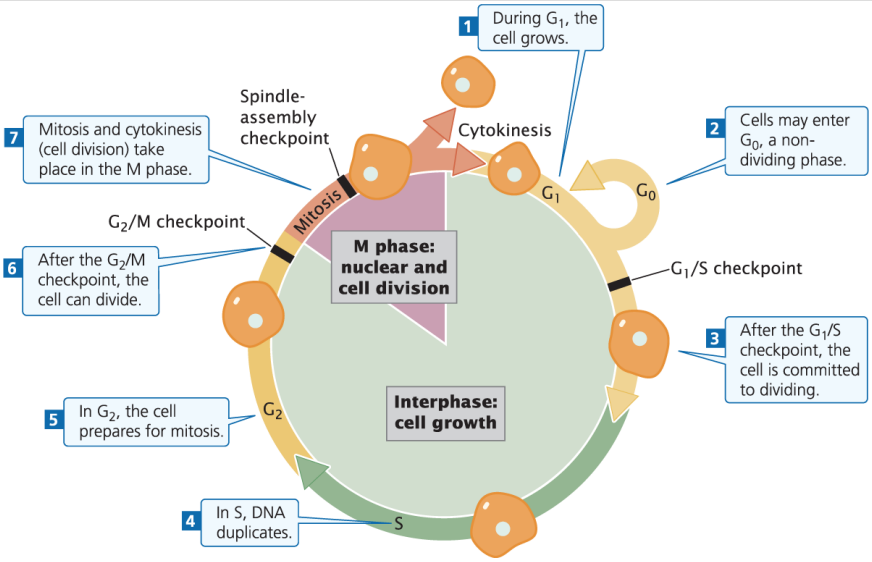
Functional chromosomes contain a centromere, telomeres, and origins of replication. The kinetochore is the multiprotein point of attachment for the spindle microtubules. Telomeres are the stabilizing ends of a chromosome. Origins of replication are sites where DNA synthesis begins. Sister chromatids are copies of a chromosome held together at the centromere.

✔ CONCEPT CHECK 3

What would be the result if a chromosome did not have a kinetochore?

The Cell Cycle and Mitosis

The **cell cycle** is the series of stages through which a cell passes from one division to the next (Figure 2.9). It is through the cell cycle that the genetic instructions for all characteristics are accurately passed from parent to daughter cells.



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2.9 The cell cycle consists of interphase (divided into three stages: G_1, S, and G_2) and the M phase.

Description

The flow chart depicts the different phases of a cell cycle in a clockwise manner. It shows a cell that is represented as a nearly circular structure with a smaller circle representing the nucleus within it. This cell enters the G subscript 1 phase. A caption reads, 1: During G subscript 1, the cell grows. A curved arrow emerges out from G subscript 1 and represents G subscript 0 stage. This arrow again bends to point towards G subscript 1

stage. A caption reads, 2: Cells may enter G subscript 0, a non-dividing phase. The cell now crosses the G subscript 1 over S checkpoint and is relatively larger in size. A caption reads, 3: after the G subscript 1 over S checkpoint, the cell is committed to dividing. The cell now enters the S phase that is represented curved forward pointing arrow. A caption emerging from this stage reads, 4: in S, D N A duplicates. The cell now enters G subscript 2 stage. A caption emerging out from G subscript 2 stage reads, 5: in G subscript 2, the cell prepares for mitosis. The cell then crosses the G subscript 2 over M checkpoint marked as a thin band over the arrow representing G subscript 2 stage. A caption reads, after the G subscript 2 over M checkpoint, the cell can divide. This major section of the circle represents the interphase. A caption reads, Interphase: Cell growth. The cell then enters the M phase which makes up the minor section of the circle. A caption reads, M phase: nuclear and cell division. The cell undergoes mitosis. A caption reads, mitosis and cytokinesis (cell division) take place in the M phase. A thin band represents spindle-assembly checkpoint. The cell crosses this checkpoint and eventually two daughter cells are produced. One of these daughter cell enters the cycle again.

The cell cycle takes place in cells that are actively dividing. A new cycle begins after a cell has divided and produced two new cells. Each new cell grows, develops, and carries out the functions specific to its cell type. At the end of the cycle, the cell divides to produce two cells, which can then undergo additional cell cycles. Progression through the cell cycle is regulated at key transition points called **checkpoints**, which allow or prohibit the cell's progression to the next stage. Checkpoints are necessary to ensure that all cellular components are present and in good working order, and to prevent cells with damaged or missing chromosomes from proliferating. Defects in checkpoints can lead to unregulated cell growth, as is seen in some cancers. The molecular basis of these checkpoints will be discussed in Chapter 23.

The cell cycle consists of two major phases. The first is **interphase**, the period between cell divisions in which the cell grows, develops, and functions. In interphase, critical events necessary for cell division also take place. The second major phase is the **M (mitotic) phase**, the period of active cell division. The M phase includes **mitosis**, the process of nuclear division, and **cytokinesis**, or cytoplasmic division. Let's take a closer look at the details of interphase and the M phase.

INTERPHASE

Interphase is the extended period of growth and development between cell divisions. Although little activity can be observed with a light microscope, the cell is quite busy: DNA is being synthesized, RNA and proteins are being produced, and hundreds of biochemical reactions necessary for cellular functions are taking place. In addition to growth and development, interphase includes several checkpoints.

By convention, interphase is divided into three stages: G_1, S, and G_2 (see Figure 2.9). Interphase begins with G_1 (for gap 1). In G_1, the cell grows and proteins necessary for cell division are synthesized; this stage typically lasts several hours. Near the end of G_1, a critical checkpoint, termed the G_1/S checkpoint, holds the cell in G_1 until the cell has all the enzymes and proteins necessary for the replication of DNA. After this checkpoint has been passed, the cell is committed to divide.

Before reaching the G_1/S checkpoint, cells may exit the active cell cycle in response to regulatory signals and pass into a nondividing phase called G_0, a stable state during which cells usually maintain a constant size. They can remain in G_0 for an extended time, even indefinitely, or they can reenter G_1 and the active cell cycle. Many cells never enter G_0; rather, they cycle continuously. On the other hand, many cells spend most of their life span in G_0.

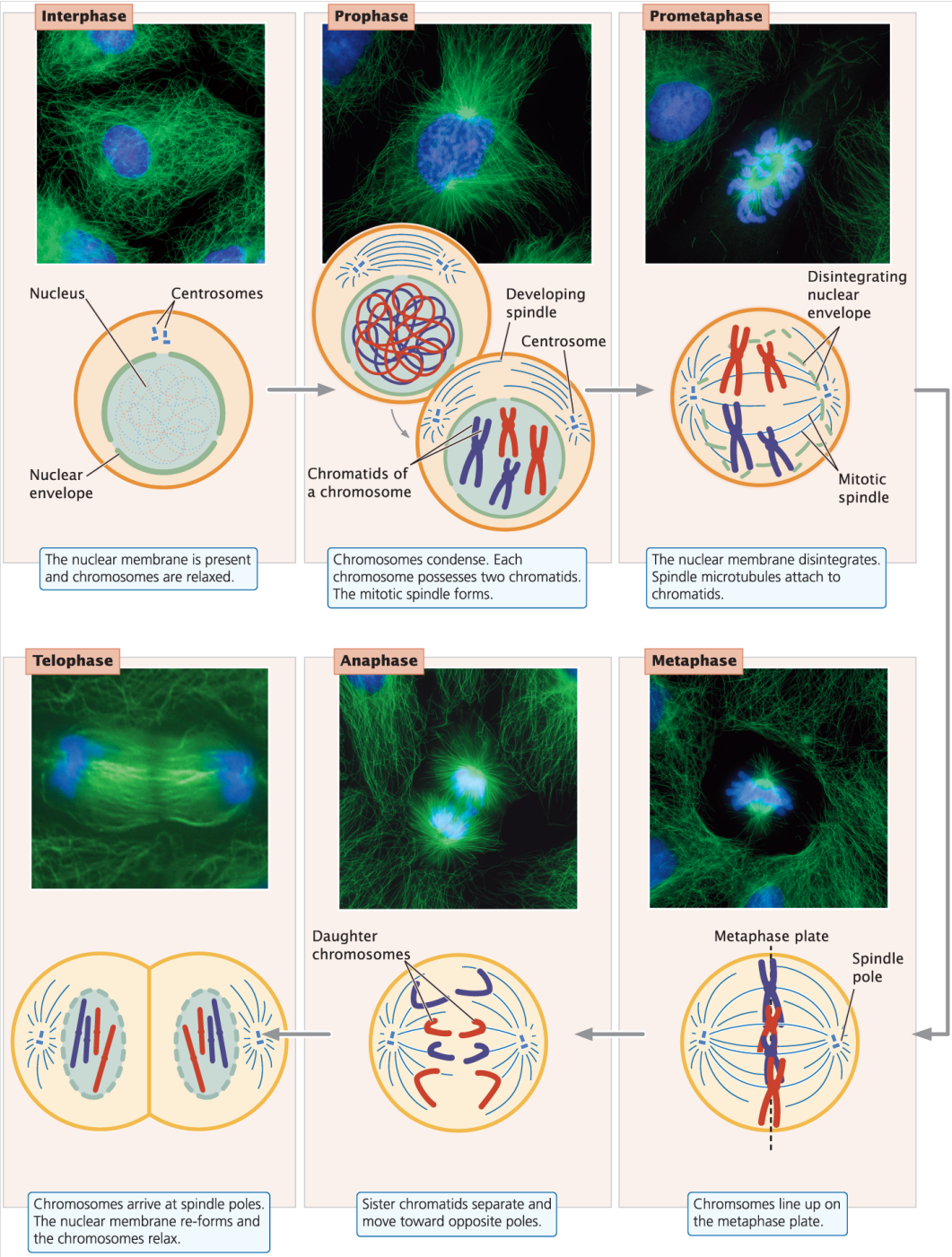
After G_1, the cell enters the S phase (for DNA synthesis), in which each chromosome is duplicated. Although the cell is committed to divide after the G_1/S checkpoint has been passed, DNA synthesis must take place before the cell can proceed to mitosis. If DNA synthesis is blocked (by drugs or by a mutation), the cell will not normally be able to undergo mitosis. Before the S phase, each chromosome is unreplicated; after the S phase, each chromosome is composed of two sister chromatids (see Figure 2.7).

After the S phase, the cell enters G_2 (gap 2). In this stage, several additional biochemical events necessary for cell division take place. The G_2/M checkpoint is reached near the end of G_2. This checkpoint is passed only if the cell's DNA is completely replicated and undamaged. Unreplicated or damaged DNA can inhibit the activation of some proteins that are necessary for mitosis to take place. After the G_2/M checkpoint has been passed, the cell is ready to divide and enters the M phase. Although the length of interphase varies from cell type to cell type, a typical dividing mammalian cell spends about 10 hours in G_1, 9 hours in S, and 4 hours in G_2 (see Figure 2.9).

Throughout interphase, the chromosomes are in a relaxed but by no means uncoiled state, and individual chromosomes cannot be seen with a microscope. This condition changes dramatically when interphase draws to a close and the cell enters the M phase.

THE M PHASE

The M phase is the part of the cell cycle in which the copies of the cell's chromosomes (sister chromatids) separate and the cell undergoes division. The separation of sister chromatids in the M phase is a critical process that results in a complete set of genetic information for each of the resulting cells. Biologists usually divide the M phase into six stages: the five stages of mitosis (prophase, prometaphase, metaphase, anaphase, and telophase), illustrated in **Figure 2.10**, and cytokinesis. It's important to keep in mind that the M phase is a continuous process and that its separation into these six stages is somewhat arbitrary.



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Photographs (top row, bottom row center, bottom row right): Dr. Torsten Wittman/Science Source; (bottom row left): Jennifer Waters/Science Source.

2.10 The cell cycle is divided into interphase and five stages of mitosis.

Description

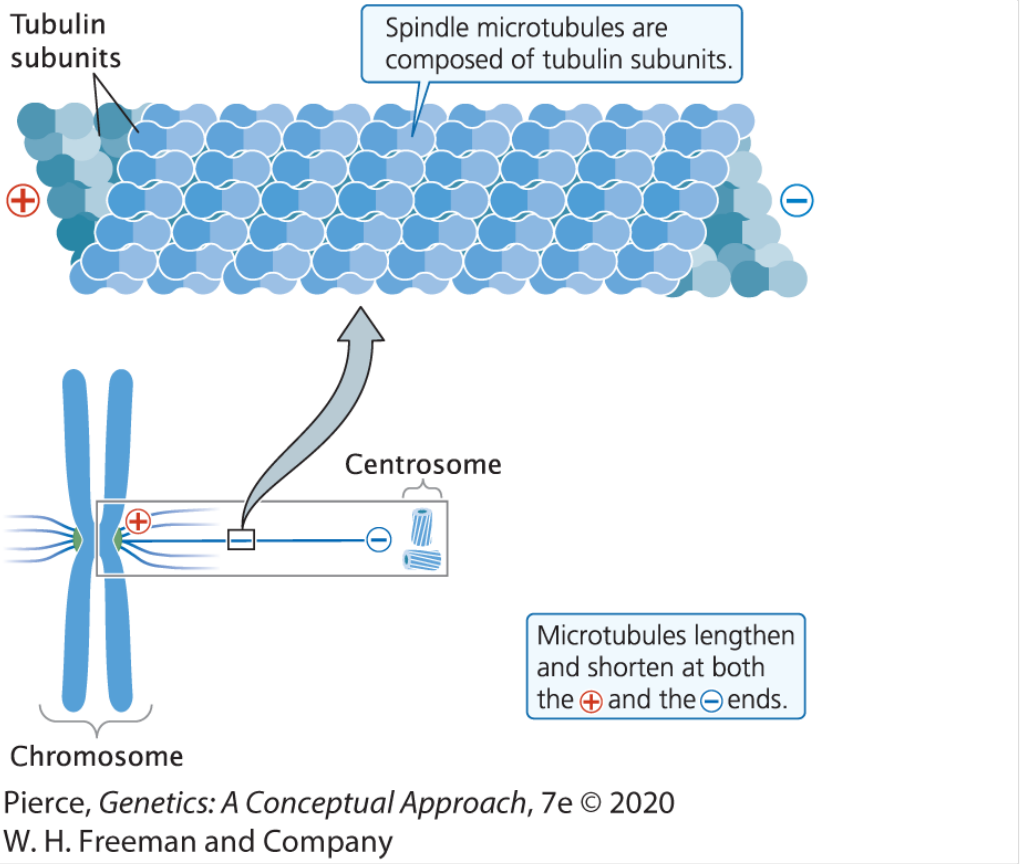
The top left box shows interphase. The nucleus with thread-like nuclear material is surrounded by a nuclear envelope and 4 small rectangular structures called centrosomes lie beside the nucleus. In the florescent image, a blue colored almost round nucleus is present with green webbed fibers around. A caption reads, the nuclear membrane is present and chromosomes are relaxed. This stage leads to prophase. The top center box shows prophase where chromosomes condense as coiled red and blue thread like structures which appear in the nucleus. The spindle fibers form as the centrosomes drift a little apart. This leads to formation of 4 X-shaped chromosomes where two are red chromosome and 2 are blue and each has 2 chromatids. A red and a blue chromosome are larger than its corresponding chromosome. The mitotic spindle elongates as the centrosomes further migrate to opposite sides of the cell. In the fluorescent image, the cell appears to have a larger nucleus (in fluorescent blue) than during interphase and the ends of the cells have spikey green material appear to be extending outwards. A caption reads, Chromosomes condense. Each chromosome possesses two chromatids. The mitotic spindle forms. This stage leads to prometaphase. Top right box shows prometaphase where the nuclear membrane disintegrates. The spindle microtubules attach to the chromatids from left and right ends and the centrosomes appear to be at opposite polar ends of the cell. The red chromosomes are at the top, attached to spindle fiber and blue ones are at bottom, as the spindle fibers now run horizontally from one cell pole to other. In the fluorescent image, a circular nucleus is not present, but blue stings project outward and all of the microtubules (in green) appear concentrated in the center of the image. A caption reads, the nuclear membrane disintegrates. Spindle microtubules attach to chromatids. This leads to next stage metaphase. The bottom right box shows metaphase where the chromosomes line up one below the other on the metaphase plate along the center of the cell. The blue one is at the top, followed by a red, blue and a red chromosome. In the fluorescent image, the blue strings that were once projecting outward, are now aligned in the middle of the green microtubules. A caption reads, chromsomes line up on the metaphase plate. This leads to next stage Anapahse. The bottom centre box shows anaphase where the sister chromatids separate as V shaped and move toward opposite poles. In the fluorescent picture, we see 2 blue and green spiky masses next to each other. A caption reads, sister chromatids separate and move toward opposite poles. This stage finally leads to next stage Telophase. The bottom right box shows telophase where the rod-shaped chromosomes arrive at spindle poles. The nuclear membrane re-forms and the chromosomes relax. Each nucleus now has 2 red(one small, one large) and 2 blue (one small, one large) rod shaped chromosomes. A vertical furrow is formed in the cell as it almost divides into two cells. One centrosome is present in each cell as tiny

spindle fibers still are attached to them. In the fluorescent image, there are 2 circular objects each touching each other, both of which contain a blue circular masses on the opposite ends and a mass of green lines, which are less green in the middle, and thus must be in the process of separating from each other. A caption reads, Chromosomes arrive at spindle poles. The nuclear membrane re-forms and the chromosomes relax.

Prophase. As a cell enters prophase, the chromosomes condense, becoming more compact and visible under a light microscope. A group of proteins called **condensins** bind to the DNA within chromosomes and bring about condensation; condensins are a type of structural maintenance of chromosomes complex, which, as previously discussed, also plays a role in chromosome segregation in bacteria. Because the chromosomes were duplicated in the preceding S phase, each chromosome consists of two sister chromatids attached at the centromere. The mitotic spindle—an organized array of microtubules that move the chromosomes in mitosis—forms. In animal cells, the spindle grows out from a pair of centrosomes that migrate to the opposite sides of the cell. Within each centrosome is a special organelle, the centriole, which is also composed of microtubules. Some plant cells do not have centrosomes or centrioles, but they do have mitotic spindles.

Prometaphase. Disintegration of the nuclear membrane marks the start of prometaphase. Spindle microtubules, which until now have been outside the nucleus, enter the nuclear region. The spindle microtubules are composed of subunits of a protein called tubulin (**Figure 2.11**). During prometaphase, tubulin molecules are added to and removed from the microtubules, causing them to undergo

repeated cycles of growth and shrinkage. When the end of a microtubule encounters a kinetochore, the microtubule becomes stabilized. Eventually, each chromosome becomes attached to microtubules from opposite spindle poles: for each chromosome, a microtubule from one of the centrosomes anchors to the kinetochore of *one* of the sister chromatids; a microtubule from the opposite centrosome then attaches to the *other* sister chromatid, anchoring the chromosome to both of the centrosomes. This arrangement is known as chromosome bi-orientation.



2.11 Microtubules are composed of tubulin subunits. Each microtubule has its plus (+) end at the kinetochore and its minus (−) end at the centrosome.

Description
At the bottom, there is a drawing of an X-shaped chromosome with 1 microtubule attached. The positive end of the microtubule is attached to the kinetochore side and the negative end is near the centrosome. The picture on top shows the tubulin subunits drawn as balls that attach in a spiral manner to form a tube-like structure. The captions read: spindle microtubules are composed of tubulin subunits. Microtubules lengthen and shorten at both the positive and the negative ends.

Metaphase. During metaphase, the chromosomes become arranged in a single plane, called the metaphase plate, between the two centrosomes. The centrosomes, now at opposite ends of the cell, with microtubules radiating outward from each one and meeting in the middle of the cell, center at the spindle poles. A spindle-assembly checkpoint ensures that each chromosome is aligned on the metaphase plate and attached to spindle microtubules from opposite poles.

Tension is generated at the kinetochore as the two conjoined chromatids are pulled in opposite directions by the spindle microtubules. This tension is required for the cell to pass through the spindle-assembly checkpoint. If one chromatid is attached to a microtubule but the other is not, no tension is generated, and the cell is unable to progress to the next stage of mitosis. The spindle-assembly checkpoint is able to detect even a single pair of chromosomes that are not properly attached to microtubules. The importance of this checkpoint is illustrated by cells that are defective in their spindle-assembly checkpoint; these cells often end up with abnormal numbers of chromosomes.

Anaphase. After the spindle-assembly checkpoint has been passed, the connection between sister chromatids breaks down and the sister chromatids separate. This separation marks the beginning of anaphase, during which the chromosomes move toward opposite spindle poles. Chromosome movement is due to the disassembly of tubulin molecules at both the kinetochore end (called the + end) and the spindle-pole end (called the − end) of the spindle microtubule (see Figure 2.11). Special proteins called molecular motors disassemble tubulin molecules and generate forces that pull the chromosome toward the spindle pole.

Telophase. After the sister chromatids have separated, each is considered a separate chromosome. Telophase is marked by the arrival of the chromosomes at the spindle poles. The nuclear membrane re-forms around each set of chromosomes, producing two separate nuclei within the cell. The chromosomes relax and lengthen, becoming indistinguishable under the light microscope. In many cells, division of the cytoplasm (cytokinesis) is simultaneous with telophase.

The major features of the cell cycle are summarized in Table 2.1. You can watch the cell cycle in motion by viewing (A) **Animation 2.1**. This interactive animation allows you to determine what happens when different processes in the cycle fail. ❯ **TRY** PROBLEM 24

TABLE 2.1 Features of the cell cycle

Stage	Major Features
G_0 phase	Stable, nondividing period of variable length.
Interphase	
G_1 phase	Growth and development of the cell; G_1/S checkpoint.
S phase	Synthesis of DNA.
G_2 phase	Preparation for division; G_2/M checkpoint.
M phase	
Prophase	Chromosomes condense and mitotic spindle forms.
Prometaphase	Nuclear membrane disintegrates, and spindle microtubules anchor to kinetochores.
Metaphase	Chromosomes align on the metaphase plate; spindle-assembly checkpoint.
Anaphase	Sister chromatids separate, becoming individual chromosomes that migrate toward spindle poles.

Telophase	Chromosomes arrive at spindle poles, the nuclear membrane re-forms, and the condensed chromosomes relax.
Cytokinesis	Cytoplasm divides; cell wall forms in plant cells.

Genetic Consequences of the Cell Cycle

What are the genetically important results of the cell cycle? From a single cell, the cell cycle produces two cells that contain the same genetic instructions. The resulting daughter cells are genetically identical with each other and with their parent cell because DNA synthesis in the S phase creates an exact copy of each DNA molecule, giving rise to two genetically identical sister chromatids. Mitosis then ensures that one of the two sister chromatids from each replicated chromosome passes into each new cell.

Another genetically important result of the cell cycle is that each of the cells produced contains a full complement of chromosomes: there is no net reduction or increase in chromosome number. Each cell also contains approximately half the cytoplasm and organelle content of the original parent cell, but no precise mechanism analogous to mitosis ensures that organelles are evenly divided.

Consequently, not all cells resulting from the cell cycle are identical in their cytoplasmic content.

THINK-PAIR-SHARE Question 2

CONCEPTS

The active cell cycle phases are interphase and the M phase. Interphase consists of G_1, S, and G_2. In G_1, the cell grows and prepares for cell division; in the S phase, DNA synthesis takes place; in G_2, other biochemical events necessary for cell division take place. Some cells enter a quiescent phase called G_0. The M phase includes mitosis and cytokinesis and is divided into prophase, prometaphase, metaphase, anaphase, and telophase. The cell cycle produces two genetically identical cells, each of which possesses a full complement of chromosomes.

✔ CONCEPT CHECK 4

Which is the correct order of stages in the cell cycle?

a. G_1, S, G_2, prophase, metaphase, anaphase
b. S, G_1, G_2, prophase, metaphase, anaphase
c. Prophase, S, G_1, G_2, metaphase, anaphase
d. S, G_1, G_2, anaphase, prophase, metaphase

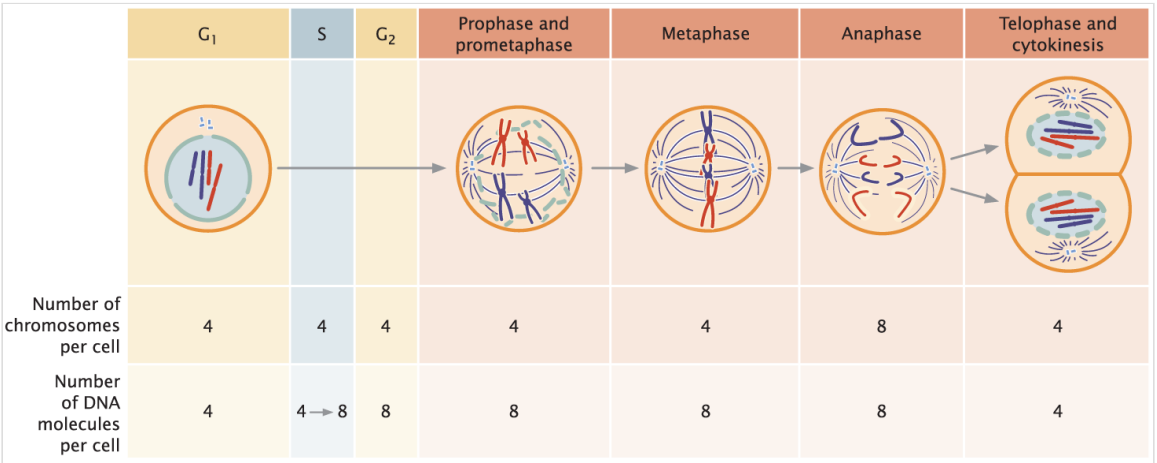
CONNECTING CONCEPTS CONNECT

Counting Chromosomes and DNA Molecules

The relations among chromosomes, chromatids, and DNA molecules frequently cause confusion. At certain times, chromosomes are unreplicated; at other times, each has undergone replication and possesses two chromatids (see Figure 2.7). Chromosomes sometimes consist of a single DNA molecule; at other times, they consist of two DNA molecules. How can we keep track of the number of these structures in the cell cycle?

There are two simple rules for counting chromosomes and DNA molecules: (1) to determine the number of chromosomes, count the number of functional centromeres; (2) to determine the number of DNA molecules, first determine if sister chromatids are present. If sister chromatids are present, the chromosome has replicated, and the number of DNA molecules is twice the number of chromosomes. If sister chromatids are not present, the chromosome has not replicated, and the number of DNA molecules is the same as the number of chromosomes.

Let's examine a hypothetical cell as it passes through the cell cycle (**Figure 2.12**). At the beginning of G_1, this diploid cell has two complete sets of chromosomes, for a total of four chromosomes. Each chromosome is unreplicated and consists of a single molecule of DNA, so there are four DNA molecules in the cell during G_1. In the S phase, each DNA molecule is copied. The two resulting DNA molecules combine with histones and other proteins to form sister chromatids. Although the amount of DNA doubles in the S phase, the number of chromosomes remains the same because the sister chromatids are tethered together and share a single functional centromere. At the end of the S phase, this cell still contains four chromosomes, each with two sister chromatids; so $4 \times 2 = 8$ DNA molecules are present.



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2.12 The number of chromosomes and the number of DNA molecules change in the course of the cell cycle. The number of chromosomes per cell equals the number of functional centromeres. The number of DNA molecules per cell equals the number of chromosomes when the chromosomes are unreplicated (no sister chromatids are present) and twice the number of chromosomes when sister chromatids *are* present.

Description

The table has 4 rows and 7 columns. The column headers from left to right are as follows: G subscript 1; S; G subscript 2; Prophase and prometaphase; Metaphase; Anaphase; and Telophase and cytokinesis. The row entries from top to bottom are as follows:

Row 1: In G subscript 1, the cell has nucleus with two pairs of rod-shaped chromosomes; one is red and the other is blue; that are not attached at the centromere. The cell then progresses directly to the fourth column representing prophase and prometaphase, skipping S and G subscript 2 column. In, prophase and prometaphase stage, the nuclear membrane disintegrates and radially arranged spindle fibers are formed from the two poles of the cell. Four X-shaped chromosomes, 2 red and 2 blue, appear at the center of the cell that are attached to their respective sister chromatid through centromere. In metaphase, these four chromosomes align in a straight line forming the metaphase plate. The spindle fibers from poles are attached to centromeres of the four chromosomes. In anaphase, sister chromatids separate thereby becoming individual chromosomes that migrate toward spindle poles. The diagram shows laterally placed V-shaped chromosomes attached to spindle fibers. In telophase and cytokinesis, the cell divides into two daughter cells wherein chromosomes arrive at spindle poles, the nuclear membrane re-forms, and the condensed chromosomes relax.

Row 2 (Number of chromosomes per cell): G subscript 1, 4; S, 4; G subscript 2, 4; Prophase and Prometaphase, 4; Metaphase, 4; Anaphase, 8; Telophase and cytokinesis, 4.

Row 3 (Number of D N A molecules per cell): G subscript 1, 4; S, 4 becomes 8; G subscript 2, 8; Prophase and Prometaphase, 8; Metaphase, 8; Anaphase, 8; Telophase and cytokinesis, 4.

Through prophase, prometaphase, and metaphase, the cell has four chromosomes and eight DNA molecules. At anaphase, however, the sister chromatids separate. Each now has its own functional centromere, so each is considered a separate chromosome. Until cytokinesis, the cell contains eight unreplicated chromosomes; thus, there are still eight DNA molecules present. After cytokinesis, the eight chromosomes (and eight DNA molecules) are distributed equally between two daughter cells, so each daughter cell contains four chromosomes and four DNA molecules—the number present at the beginning of the cell cycle.

In summary, the number of chromosomes increases only in anaphase, when the two chromatids of a chromosome separate and become distinct chromosomes. The number of chromosomes decreases only through cytokinesis. The number of DNA molecules increases only in the S phase and decreases only through cytokinesis.

2.3 Sexual Reproduction Produces Genetic Variation Through the Process of Meiosis

If all reproduction were accomplished through mitosis, life would be quite dull because mitosis produces only genetically identical progeny. With only mitosis, you, your children, your parents, your brothers and sisters, your cousins, and many people whom you don't even know would be clones—copies of one another. Only the occasional mutation would introduce any genetic variation. All organisms reproduced in this way for the first 2 billion years of Earth's existence (and some organisms still do today). Then, about 1.5 billion to 2 billion years ago, something remarkable evolved: cells that produce genetically variable offspring through sexual reproduction.

The evolution of sexual reproduction is among the most significant events in the history of life. As will be discussed in Chapters 24 and 25, the pace of evolution depends on the amount of genetic variation present. By shuffling the genetic information from two parents, sexual reproduction greatly increases the amount of genetic variation and allows for accelerated evolution. Most of the tremendous diversity of life on Earth is a direct result of sexual reproduction.

Sexual reproduction consists of two processes. The first is **meiosis**, which leads to gametes in which the number of chromosomes is reduced by half. The second process is **fertilization**, in which two haploid gametes fuse and restore the number of chromosomes to its original diploid value.

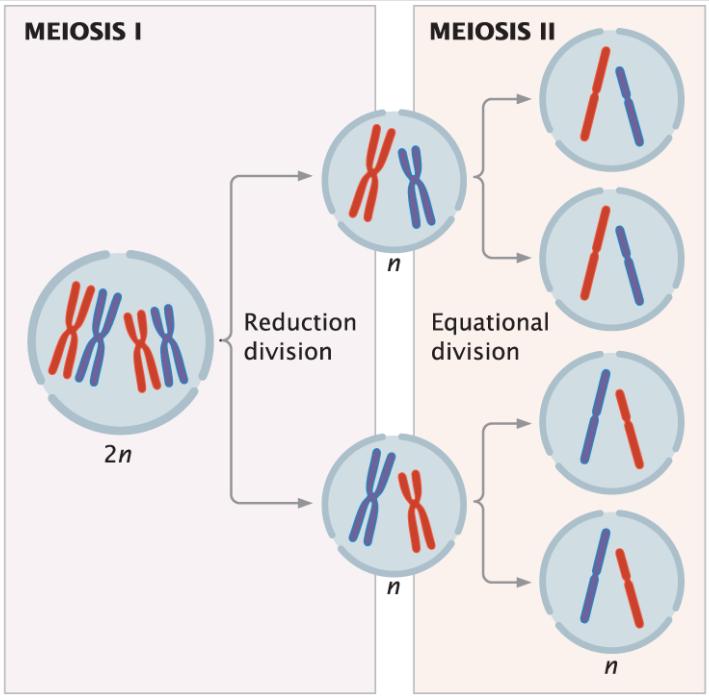
Meiosis

The words *mitosis* and *meiosis* are sometimes confused. They sound a bit alike and may appear superficially similar. But don't be deceived. The outcomes of mitosis and meiosis are radically different, and several unique events that have important genetic consequences take place only in meiosis.

How does meiosis differ from mitosis? Mitosis consists of a single nuclear division and is usually accompanied by a single cell division. Meiosis, on the other hand, consists of two divisions. After mitosis, the chromosome number in the newly formed cells is the same as that in the original cell, whereas meiosis causes the chromosome number in the newly formed cells to be reduced by half. Finally, mitosis produces genetically identical cells, whereas meiosis produces genetically variable cells. Let's see how these differences arise.

Like mitosis, meiosis is preceded by an interphase that includes G_1, S, and G_2 stages. Meiosis consists of two distinct processes: meiosis I

and meiosis II, each of which includes a cell division. The first division, which comes at the end of meiosis I, is termed the reduction division because the number of chromosomes per cell is reduced by half (**Figure 2.13**). The second division, which comes at the end of meiosis II, is sometimes termed the equational division. The events of meiosis II are similar to those of mitosis. However, meiosis II differs from mitosis in that chromosome number has already been halved in meiosis I, so the cell does not begin with the same number of chromosomes as it does in mitosis (see Figure 2.13).



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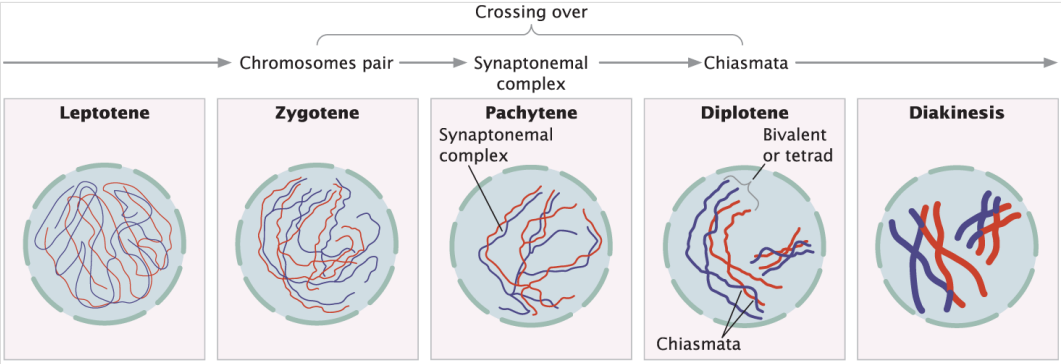
2.13 Meiosis includes two cell divisions. In this illustration, the original cell is diploid (2*n*) with four chromosomes. After two meiotic divisions, each resulting cell is haploid (1*n*) with two chromosomes.

MEIOSIS I

During interphase, the chromosomes are relaxed and indistinguishable as diffuse chromatin. **Prophase I** is a lengthy stage, divided into five substages (**Figure 2.14**). In leptotene, the chromosomes condense and become visible. In zygotene, the chromosomes continue to condense; homologous chromosomes pair up and begin **synapsis**, a very close pairing association. Each homologous pair of synapsed chromosomes, called a **bivalent** or **tetrad**, consists of four chromatids. In pachytene, the chromosomes become shorter and thicker, and a three-part synaptonemal complex develops between homologous chromosomes. The function of the synaptonemal complex is unclear, but the chromosomes of many cells deficient in this complex do not separate properly.



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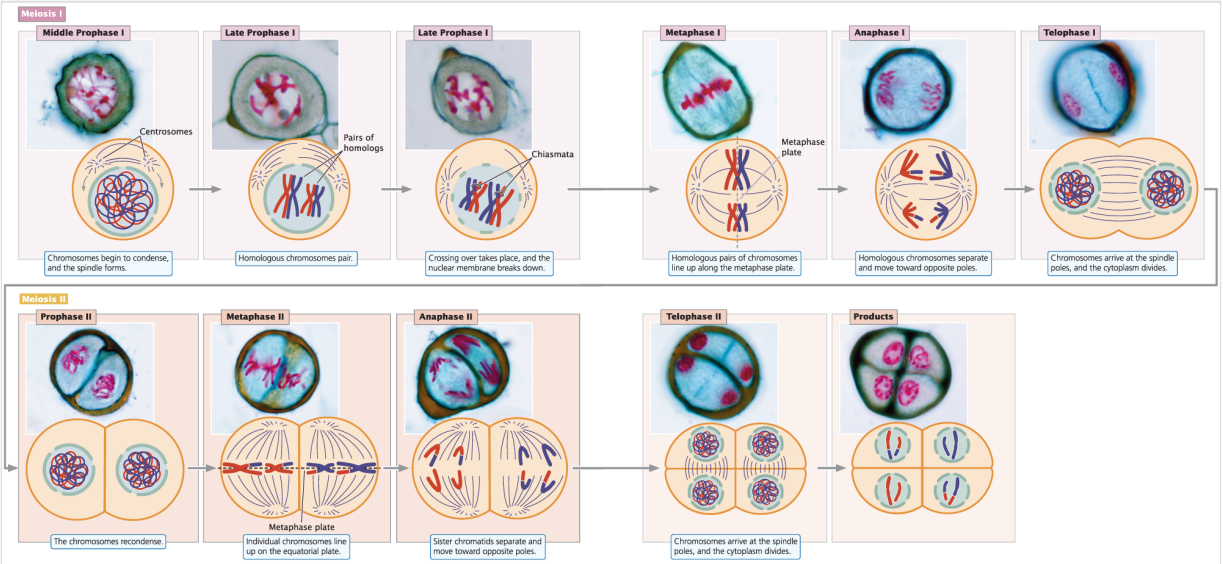
2.14 Substages of prophase I. Crossing over takes place during prophase I.

Crossing over, in which homologous chromosomes exchange genetic information, takes place in prophase I. Crossing over generates genetic variation (as we will see shortly) and is essential

for the proper alignment and separation of homologous chromosomes. Each location where two chromosomes cross is called a *chiasma* (plural, *chiasmata*). In diplotene, the centromeres of the paired chromosomes move apart, but the two homologs remain attached at each chiasma. During diakinesis, the final substage of prophase I, chromosome condensation continues, the nuclear membrane breaks down and the spindle forms, setting the stage for metaphase I. The stages of meiosis are outlined in Figure 2.15.



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University of Wisconsin Plant Teaching Collection, photographs by Michael Clayton.

2.15 Meiosis is divided into stages.

Description

In the first panel at top left with the heading metaphase 1, the homologous pairs of X-shaped chromosomes line up along the metaphase plate one below the other. A large red chromosome with blue part is paired with large blue chromosome with a red part. Below this, a small red X shaped chromosome is paired with small blue X shaped chromosome. The spindle fibers run radially from one cell pole to other. In the corresponding cell staining, the D N A is condensed and located in the middle of the cell. A caption reads, “Homologous pairs of chromosomes line up along the metaphase plate.”

In second panel at top center with the heading anaphase 1, the homologous chromosomes, which have crossed-over and contain a different combination of genes, indicated by different patterns of red and blue, separate and move toward the opposite poles. The red colored chromosomes with blue part move towards left and the blue chromosomes with red part move towards right. In the stained cell image, we see squiggly lines of D N A located at opposite ends of the cell. A caption reads, “Homologous chromosomes separate and move toward opposite poles.”

In the third panel at top right with the heading telophase 1, the chromosomes arrive at the spindle poles and the cytoplasm divides as a furrow forms in between. Both the halves have a nucleus with red and blue thin strings coiled. The spindle fibers run from one nucleus to the other. In the cell staining, a cell divided into two cells with each having nuclei located on opposite ends and a blue ridge forming in the center. A caption reads, “Chromosomes arrive at the spindle poles and the cytoplasm divides.”

Fourth panel at bottom left depicts the beginning of telophase 2 where the chromosomes arrive at the spindle poles and the cytoplasm divides into 4 almost equal halves. Each half has a nucleus with blue and red thin string like coiled chromosome material. Spindle fibers run vertically from one cell to other from top to bottom. In the stained cell image, one cell has 2 circular sections, each having two condensed nuclei at opposite ends of the cell. A blue ridge appears to be forming in the middle of the cell. A caption reads, “Chromosomes arrive at the spindle poles and the cytoplasm divides.”

In the last panel at bottom center, the cell divisions occur and 4 cells are formed as products. One cell has all red rod shaped D N A, one has all blue rod shaped D N A, and two cells have hybrids: one cell has a large and a small red rod shaped D N A both with a blue part and the other cell has a large and a small blue rod shaped D N A both with a red part. In the cell staining diagram, a cell is divided into 4 halves with nearly circular red D N A in all four parts.

For meiosis 1: In panel 1 at the top left, middle prophase 1 is depicted. At this stage, the chromosomes begin to condense which are depicted as coiled red and blue strings. The spindle fibers form outside the nucleus, with the centrosomes migrating to opposite ends of the cell. In the cell staining, the nucleus appears to contain D N A (stained in red) distributed throughout the nucleus (colored green). A caption reads, “Chromosomes begin to condense, and the spindle forms.” This leads to next stage, late prophase 1.

In panel 2 at top center with the heading late prophase 1, the homologous chromosomes pair up. A large red X shaped chromosome pairs up with large blue X shaped chromosome and a small red X-shaped chromosome pairs up with a small blue X-shaped chromosome. The spindle fibers elongate as the centrosomes drift farther away. In the cell staining, the red masses start to clump in the nucleus. A caption reads, “Homologous chromosomes pair.”

In panel 3 at top right with the heading late prophase 1, crossing over takes place, shown by the legs of a blue and a red X-shaped chromosome touching at the chiasmata and the colors of the legs switching. The nuclear membrane also starts to break down. In the cell staining, the red masses almost are distributed throughout the nucleus. A caption reads, crossing over takes place, and the nuclear membrane breaks down.

For meiosis 2: In panel 1 at bottom left with the heading prophase 2, the chromosomes recondense. The cell is divided into two vertical halves. Both the cells have individual nucleus with red and blue coiled string like chromosome material within them. In the cell staining, the cell is divided into two halves and both have red colored D N A condensed and centrally localized. A caption reads, “The chromosomes recondense.”

In panel 2 at bottom center with the heading metaphase 2, the individual chromosomes line up horizontally on the metaphase plate. The X-shaped chromosomes are hybrid in color, containing different amounts of red and blue. The spindle fibers run vertically from one end to other end. In the cell staining, a cell shows 2 almost equals regions, each having red, thick string shaped D N A aligned in the center. A caption reads, individual chromosomes line up on the equatorial plate.

In panel 3 at bottom right with the heading anaphase 2, the sister chromatids containing hybrid D N A separate and move toward opposite poles. In the left half, hybrid red chromatids with blue parts are pulled upwards and red chromatids are pulled downwards. In the right half, hybrid blue chromatids with red parts are pulled downwards and blue chromatids are pulled upwards. In the cell staining, there are 2 circular regions inside the cell, each having a mass of red D N A on opposite ends of the nuclei. A caption reads, sister chromatids separate and move toward opposite poles.

Metaphase I is initiated when homologous pairs of chromosomes align along the metaphase plate (see Figure 2.15). A microtubule

from one spindle pole attaches to one chromosome of a homologous pair, and a microtubule from the other pole attaches to the other member of the pair. **Anaphase I** is marked by the separation of homologous chromosomes. The two chromosomes of a homologous pair are pulled toward opposite poles. Although the homologous chromosomes separate, the sister chromatids remain attached and travel together. In **telophase I**, the chromosomes arrive at the spindle poles, and the cytoplasm divides.

MEIOSIS II

The period between meiosis I and meiosis II is **interkinesis**, in which the nuclear membrane re-forms around the chromosomes clustered at each pole, the spindle breaks down, and the chromosomes relax. The cells then pass through **prophase II**, in which the events of interkinesis are reversed: the chromosomes recondense, the spindle re-forms, and the nuclear membrane once again breaks down. In interkinesis in some types of cells, the chromosomes remain condensed and the spindle does not break down. These cells move directly from cytokinesis into **metaphase II**, which is similar to metaphase of mitosis: the replicated chromosomes line up on the metaphase plate, with the sister chromatids facing opposite poles.

In **anaphase II**, the sister chromatids separate, and the chromatids are pulled to opposite poles. Each chromatid is now a distinct chromosome. In **telophase II**, the chromosomes arrive at the spindle

poles, a nuclear membrane re-forms around the chromosomes, and the cytoplasm divides. The chromosomes relax and are no longer visible. ❯ **TRY** PROBLEM 29

THINK-PAIR-SHARE Questions 3, 4

CONCEPTS

Meiosis consists of two distinct processes: meiosis I and meiosis II. Meiosis I includes the reduction division, in which homologous chromosomes separate and chromosome number is reduced by half. In meiosis II (the equational division), sister chromatids separate.

✔ CONCEPT CHECK 5

Which of the following events takes place in metaphase I?

a. Crossing over occurs.
b. Chromosomes condense.
c. Homologous pairs of chromosomes line up on the metaphase plate.
d. Individual chromosomes line up on the metaphase plate.

The major events of meiosis are summarized in Table 2.2. To examine the details of meiosis and the consequences of its failure, take a look at (A) **Animation 2.2.**

TABLE 2.2 Major events in each stage of meiosis

Stage	Major Features
Meiosis I	

Prophase I	Chromosomes condense, homologous chromosomes synapse, crossing over takes place, the nuclear membrane breaks down, and the mitotic spindle forms.
Metaphase I	Homologous pairs of chromosomes line up on the metaphase plate.
Anaphase I	The two chromosomes (each with two chromatids) of a homologous pair separate and move toward opposite poles.
Telophase I	Chromosomes arrive at the spindle poles.
Cytokinesis	The cytoplasm divides to produce two cells, each having half the original number of chromosomes.
Interkinesis	In some types of cells, the spindle breaks down, chromosomes relax, and a nuclear membrane re-forms, but no DNA synthesis takes place.
Meiosis II	
Prophase II*	Chromosomes condense, the spindle forms, and the nuclear membrane disintegrates.
Metaphase II	Individual chromosomes line up on the

	metaphase plate.
Anaphase II	Sister chromatids separate and move as individual chromosomes toward opposite spindle poles.
Telophase II	Chromosomes arrive at the spindle poles; the spindle breaks down and a nuclear envelope re-forms.
Cytokinesis	The cytoplasm divides.

*Only in cells in which the spindle has broken down, chromosomes have relaxed, and the nuclear membrane has re-formed in telophase I. Other types of cells proceed directly to metaphase II after cytokinesis.

Sources of Genetic Variation in Meiosis

What are the overall consequences of meiosis? First, meiosis comprises two divisions, so each original cell produces four cells (although there are exceptions to this generalization, as, for example, in many female animals; see Figure 2.20b). Second, chromosome number is reduced by half, so cells produced by meiosis are haploid. Third, cells produced by meiosis are genetically different from one another and from the parent cell. These genetic differences result from two processes that are unique to meiosis: crossing over and the random separation of homologous chromosomes.

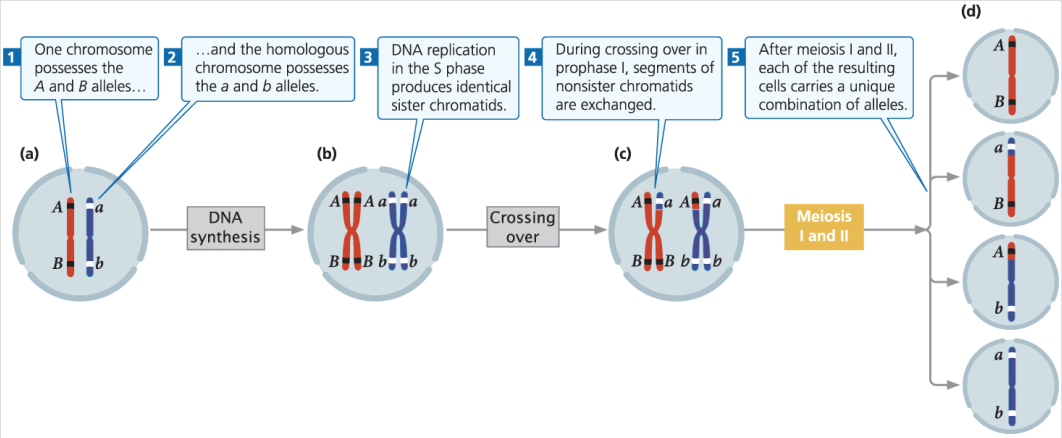
CROSSING OVER

Crossing over, which takes place in prophase I, refers to the exchange of genetic material between nonsister chromatids (chromatids from different homologous chromosomes). Evidence from yeast suggests that crossing over is initiated in zygotene, before the synaptonemal complex develops, and is not completed until near the end of prophase I (see Figure 2.14). In other organisms, crossing over is initiated after the formation of the synaptonemal complex; in still others, there is no synaptonemal complex.

After crossing over has taken place, the sister chromatids are no longer identical. Crossing over is the basis for intrachromosomal **recombination**, the creation of new combinations of alleles on a chromatid. To see how crossing over produces genetic variation, consider two pairs of alleles, which we will abbreviate *Aa* and *Bb*. Assume that one chromosome possesses the *A* and *B* alleles and its homolog possesses the *a* and *b* alleles (**Figure 2.16a**). When DNA is replicated in the S phase, each chromosome duplicates, and so the resulting sister chromatids are identical (**Figure 2.16b**).

In the process of crossing over, there are breaks in the DNA strands, and those breaks are repaired in such a way that segments of nonsister chromatids are exchanged (**Figure 2.16c**). The molecular basis of this process will be described in more detail in Chapter 12. The important thing here is that after crossing over has taken place, the two sister chromatids are no longer identical: one chromatid has

alleles *A* and *B*, whereas its sister chromatid (the chromatid that underwent crossing over) has alleles *a* and *B*. Likewise, one chromatid of the other chromosome has alleles *a* and *b*, and the other chromatid has alleles *A* and *b*. Each of the four chromatids now carries a unique combination of alleles: *A B*, *a B*, *A b*, and *a b*. Eventually, the two homologous chromosomes separate, each going into a different cell. In meiosis II, the two chromatids of each chromosome separate, and thus each of the four cells resulting from meiosis carries a different combination of alleles (Figure 2.16d). You can see how crossing over affects genetic variation by viewing **Animation 2.3**.



2.16 Crossing over produces genetic variation.

Description

The illustration “a” on the left shows a nucleus wherein one rod-shaped chromosome (in red) possesses the big A and big B alleles, and its homologous rod-shaped chromosome (in blue) possesses the small A and small B alleles. A caption reads, one chromosome possesses the big A and big B alleles and the homologous chromosome possesses the small A and small B alleles. D N A synthesis occurs and illustration “b” shows identical

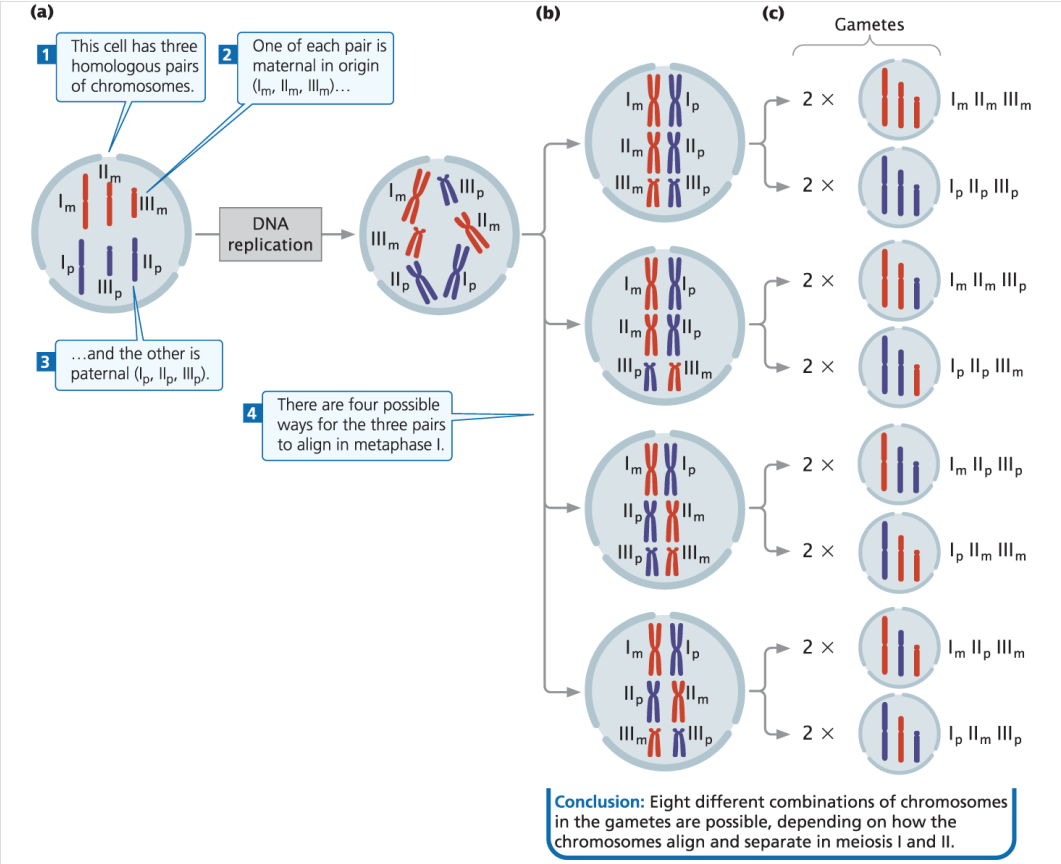
sister chromatids are produced. Now, two X-shaped chromosomes are produced where one is blue and other is red. A caption reads, D N A replication in the S phase produces identical sister chromatids. Crossing over occurs and illustration “c” shows exchange of segments between non-sister chromatids. X-shaped chromosome in red has exchanged a small part of the end of one of its chromatids and is now partially blue containing the small A gene. The blue chromosome now has a small part of red chromosome with gene big A. A caption reads, during crossing over in prophase 1, segments of nonsister chromatids are exchanged. Meiosis 1 and 2 occur, and illustration “d” shows 4 resulting cells that carry a unique combination of alleles: one is red rod shaped having genes big A and big B, one is blue rod shaped having small A and small B genes, one is mostly red rod shaped with a blue end having big B and small A genes, and one is mostly blue rod shaped with a red end, having big A and small B genes.

RANDOM SEPARATION OF HOMOLOGOUS CHROMOSOMES

The second process of meiosis that contributes to genetic variation is the random distribution of chromosomes in anaphase I after their random alignment in metaphase I. To illustrate this process, consider a cell with three pairs of chromosomes, I, II, and III (Figure 2.17a). One chromosome of each pair is maternal in origin (I_m, II_m, and III_m); the other is paternal in origin (I_p, II_p, and III_p). The chromosome pairs line up in the center of the cell in metaphase I, and in anaphase I, the chromosomes of each homologous pair separate. Note that each cell gets one chromosome from each of the three pairs (I, II, and III).

How each pair of homologs aligns and separates is random and independent of how other pairs align and separate (Figure 2.17b). By

chance, all the maternal chromosomes might migrate to one side and all the paternal chromosomes to the other. After division, one cell would contain chromosomes I_m, II_m, and III_m, and the other, I_p, II_p, and III_p. Alternatively, the I_m, II_m, and III_p chromosomes might move to one side and the I_p, II_p, and III_m chromosomes to the other. These different migration patterns would produce different combinations of chromosomes in the resulting cells (**Figure 2.17c**). There are four ways in which the chromosomes in a diploid cell with three homologous pairs can migrate, producing a total of eight different combinations of chromosomes in the gametes. In general, the number of possible combinations is 2^n, where n equals the number of homologous pairs. As the number of chromosome pairs increases, the number of combinations quickly becomes very large. In humans, who have 23 pairs of chromosomes, 2^{23}, or 8,388,608, different combinations of chromosomes are made possible by the random separation of homologous chromosomes. You can explore the random distribution of chromosomes by viewing **Animation 2.3**. The genetic consequences of this process, termed independent assortment, will be explored in more detail in Chapter 3.



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2.17 The random distribution of chromosomes in meiosis produces genetic variation. In this example, the cell possesses three homologous pairs of chromosomes.

Description

Illustration ‘a’ on the extreme left shows a cell with three rod-shaped homologous pairs of chromosomes named 1 subscript m, 1 subscript p; 2 subscript m, 2 subscript p; and 3 subscript m, 3 subscript p. Length of the chromosomes shortens from 1 through 3 in both the colors. The chromosomes with labeling 1 subscript m through 3 subscript m are red, and with labeling 1 subscript p through 3 subscript p are blue in color. First caption reads, 1: this cell has three homologous pairs of chromosomes. Second caption reads, 2: one of each pair is maternal in origin (1 subscript m, 2 subscript m, and 3 subscript m). The maternal origin chromosomes are represented in red. Third caption reads, 3: and the other is paternal (1 subscript p, 2 subscript p, and 3 subscript p). The paternal origin chromosomes are represented in blue. D N A replication occurs and the cell now has six

X-shaped chromosomes namely: 1 subscript m, 3 subscript p, 3 subscript m, 2 subscript m, 2 subscript p, and 1 subscript p. An arrow from this cell leads to illustration “b”.

Illustration b in the center shows four possible ways in which the three chromosome pairs can align in metaphase. 1, Illustration c on the extreme right shows eight different possible combinations of chromosomes in the gametes, depending on how the chromosomes align and separate in meiosis 1 and 2, in the first case, 1 subscript m and 1 subscript p are aligned at the top, 2 subscript m and 2 subscript p are aligned at the center, 3 subscript m and 3 subscript p are aligned at the bottom. This can lead to 2 gametes with 1 subscript m, 2 subscript m, and 3 subscript m and 2 gametes with 1 subscript p, 2 subscript p, and 3 subscript p. In the second case, 1 subscript m and 1 subscript p are aligned at the top, 2 subscript m and 2 subscript m at the center; and 3 subscript p and 3 subscript m aligned at the bottom. This can lead to 2 gametes with 1 subscript m, 2 subscript m, and 3 subscript p and 2 gametes with 1 subscript p, 2 subscript p, and 3 subscript m. In the third case, 1 subscript m, 1 subscript p are aligned at the top, 2 subscript p and 2 subscript m are aligned at the centre, and 3 subscript p and 3 subscript m are aligned at the bottom. This can lead to 2 gametes with 1 subscript m, 2 subscript p, and 2 subscript p and 2 gametes with 1 subscript p, 2 subscript m, and 2 subscript m. In the fourth case, 1 subscript m and 1 subscript p are aligned at the top, 2 subscript p and 2 subscript m are at the center, and 3 subscript m and 3 subscript p are aligned at the bottom. This may lead to 2 gametes with 1 subscript m, 2 subscript p, and 3 subscript m and 2 gametes with 1 subscript p, 2 subscript m, and 3 subscript p. The conclusion states that eight different combinations of chromosomes in the gametes are possible, depending on how the chromosomes align and separate in meiosis 1 and 2.

In summary, crossing over shuffles alleles on the *same* chromosome into new combinations, whereas the random distribution of maternal and paternal chromosomes shuffles alleles on *different* chromosomes into new combinations. Together, these two processes are capable of producing tremendous amounts of genetic variation among the cells resulting from meiosis. **› TRY PROBLEMS 37 AND 38**

THINK-PAIR-SHARE Question 5

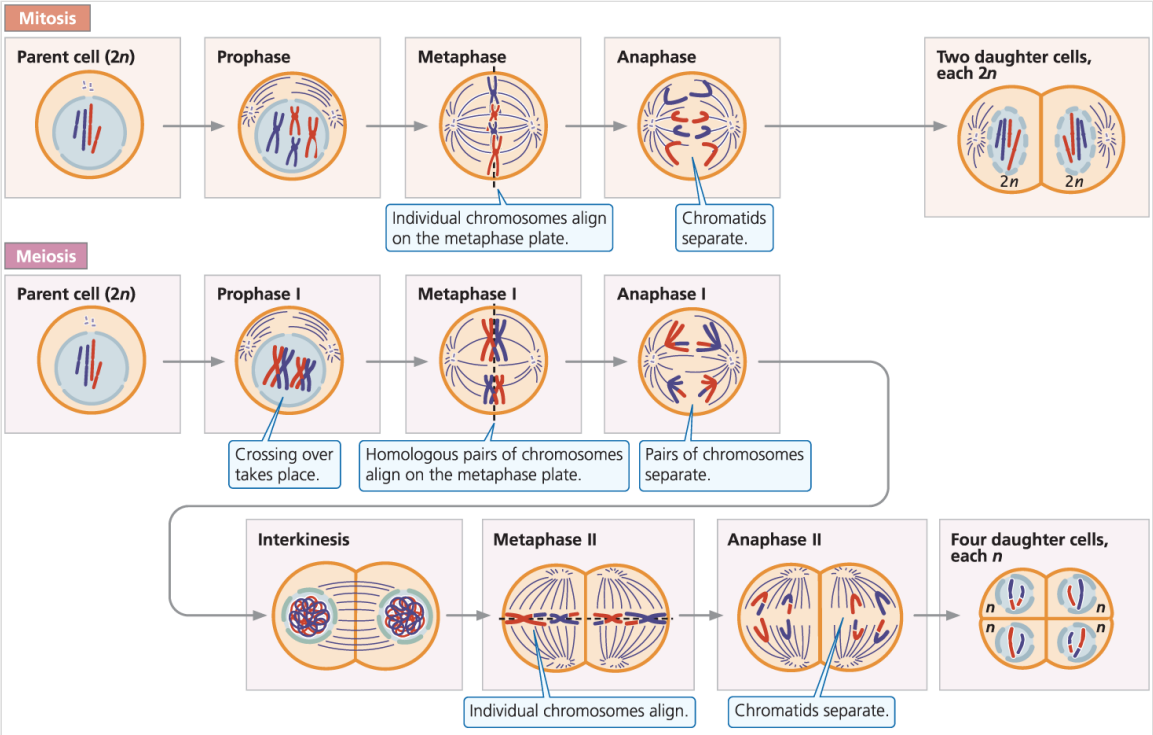
CONCEPTS

The two mechanisms that produce genetic variation in meiosis are crossing over and the random separation of maternal and paternal chromosomes into gametes.

CONNECTING CONCEPTS CONNECT

Mitosis and Meiosis Compared

Now that we have examined the details of mitosis and meiosis, let's compare the two processes (**Figure 2.18** and **Table 2.3**). In both mitosis and meiosis, the chromosomes condense and become visible; both processes include the movement of chromosomes toward the spindle poles, and both are accompanied by cell division. Beyond these similarities, the processes are quite different.



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2.18 Mitosis and meiosis compared.

Description

The first picture in both mitosis and meiosis shows a parent cell with a 2 n nucleus. The nucleus has 4 rod shaped chromosomes where 2 are red (1 small, 1 large) and 2 are blue (1 small, 1 large). The second picture shows the cell in prophase in mitosis and prophase 1 in meiosis, when there are 2 sets of intact X-shaped chromosomes (two red chromosomes where one chromosome is smaller than other and two blue chromosomes where one chromosome is smaller than other). Spindle fibers are produced over the nucleus as the centrosomes drift apart. No crossing over takes place in mitosis and 4 chromosomes are arranged randomly. In the picture below for meiosis, crossing over is occurring at this stage as larger blue and larger red chromosomes overlap and smaller red and smaller blue chromosomes overlap. A caption reads, crossing over takes place.

In the metaphase set of pictures, the X-shaped chromosomes align individually in the middle of the cell one below the other; attached to the spindle fibers running from one cell pole to other during mitosis. The large blue chromosome is at top followed by small red, small blue and finally large red. A caption reads, individual chromosomes align on the metaphase plate. In the picture below, during meiosis they align in groups. The large red and large blue attach together at top followed by small blue and small red at the bottom. A caption reads, homologous pairs of chromosomes align on the metaphase plate.

In the anaphase set of pictures, in mitosis, single chromatids are being pulled to opposite ends of the cell as V-shaped chromatids. A caption reads, chromatids separate. During meiosis, the X-shaped chromosomes wholly move to opposite sides and also have a part of other colored chromosome due to crossing over. A caption reads, pairs of chromosomes separate.

The next set of pictures, starting with interkinesis, only occurs in meiosis. During interkinesis, the cell almost divides into two halves but is still attached together. Both have chromosome material inside individual nuclear envelopes. Spindle fibers are attached between both these cells while a clear vertical division appears between both the cells.

In the metaphase 2 and anaphase 2 pictures during meiosis, the arrangements in the nucleus in meiosis are more similar to metaphase and anaphase of mitosis but here the X-shaped chromosomes align horizontally in the middle of the cell and the individual chromatids are pulled apart respectively. The spindle fibers are now aligned from top to bottom and not horizontally as in case of mitosis.

Two captions read, Individual chromosomes align. Chromatids separate.

The final pictures show the results of mitosis and meiosis. For mitosis, there are 2 daughter cells, each with the same type of nucleus, 2 n, and same combination of genes: 2 red (one small, one large) rod shaped chromatids and 2 blue (one small, one large) rod shaped chromatids. The cell is divided vertically into two halves and one centrosome with tiny spindle fibers appear in each cell.

The results for meiosis are 4 daughter cells, each with an n nucleus. The cell is divided into 4 almost equal halves. Two daughter cells have hybrid chromatids while two have normal copies.

TABLE 2.3 Comparison of mitosis, meiosis I, and meiosis II

Event	Mitosis	Meiosis I	Meiosis II
Cell division	Yes	Yes	Yes
Reduction in chromosome number	No	Yes	No
Genetic variation produced	No	Yes	No
Crossing over	No	Yes	No
Random distribution of maternal and paternal chromosomes	No	Yes	No
Metaphase	Individual	Homologous	Individual

	chromosomes line up	pairs line up	chromosomes line up
Anaphase	Chromatids separate	Homologous chromosomes separate	Chromatids separate

Mitosis results in a single cell division and usually produces two daughter cells. Meiosis, in contrast, comprises two cell divisions and usually produces four cells. In diploid cells, homologous chromosomes are present before both meiosis and mitosis, but the pairing of homologs takes place only in meiosis.

Another difference is that in meiosis, chromosome number is reduced by half as a consequence of the separation of homologous pairs of chromosomes in anaphase I, but no chromosome reduction takes place in mitosis. Furthermore, meiosis is characterized by two processes that produce genetic variation: crossing over (in prophase I) and the random distribution of maternal and paternal chromosomes (in anaphase I). There are normally no equivalent processes in mitosis.

Mitosis and meiosis I also differ in the behavior of chromosomes in metaphase and anaphase and their outcomes. In metaphase I of meiosis, *homologous pairs* of chromosomes line up on the metaphase plate, whereas *individual chromosomes* line up on the metaphase plate in metaphase of mitosis (and in metaphase II of meiosis). In anaphase I of meiosis, *paired chromosomes* separate and migrate toward opposite spindle poles, each chromosome possessing two chromatids attached at the centromere. In contrast, in anaphase of mitosis (and in anaphase II of meiosis), *sister chromatids* separate, and each chromosome that moves toward a spindle pole is unreplicated. Therefore, in contrast with both mitosis and meiosis II, meiosis I produces nonidentical daughter cells. **› TRY PROBLEMS 31 AND 32**

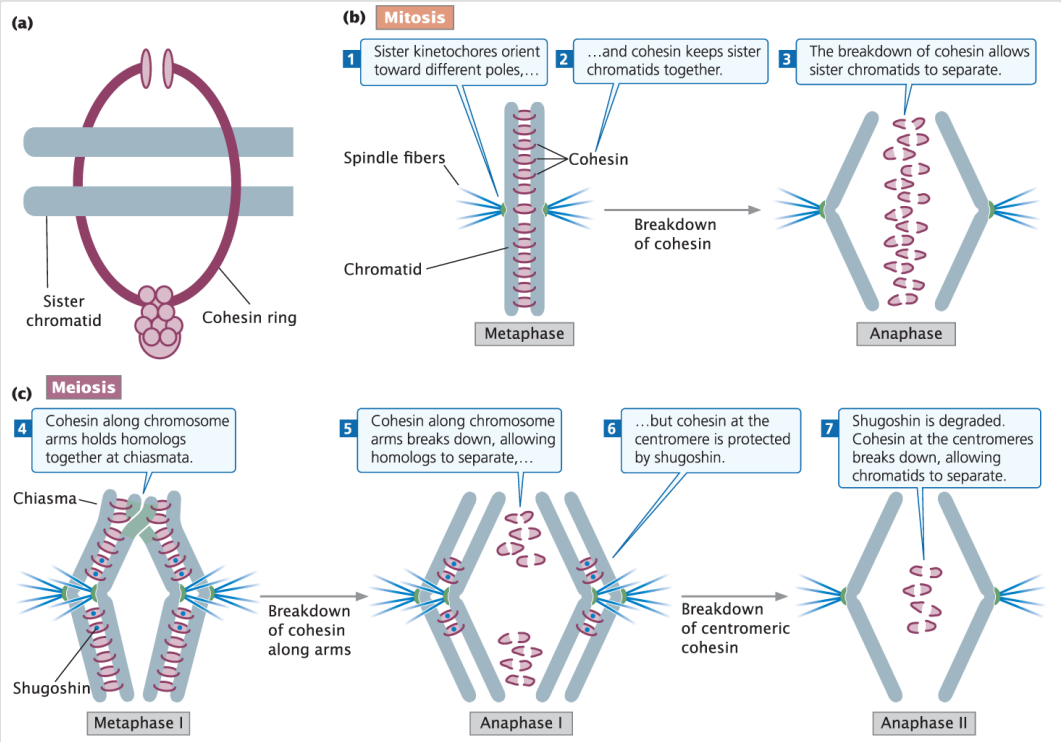
THINK-PAIR-SHARE Questions 6 and 7

The Separation of Sister Chromatids and Homologous Chromosomes

Some of the molecules required for the joining and separation of chromatids and homologous chromosomes have been identified. **Cohesin**, a protein that holds chromatids together, is key to the behavior of chromosomes in mitosis and meiosis (**Figure 2.19a**). Cohesin is another type of structural maintenance of chromosomes (SMC) complex, related to condensins and SMCs that help bring about chromosome condensation in eukaryotes and chromosome segregation in bacteria. Cohesin has a ring-like structure that encircles and holds together the sister chromatids (**Figure 2.19a**). It is loaded onto the chromosome in the S phase and persists through G_2 and early mitosis. In anaphase of mitosis, cohesin along the entire length of the chromosome is broken down by an enzyme called separase, allowing the sister chromatids to separate **(Figure 2.19b**).

As we have seen, mitosis and meiosis differ fundamentally in the behavior of chromosomes in anaphase (see Figure 2.18). Why do homologs separate in anaphase I of meiosis, whereas chromatids separate in anaphase of mitosis and anaphase II of meiosis? It is important to note that the forms of cohesin used in mitosis and meiosis differ. At the beginning of meiosis, the meiosis-specific cohesin is found along the entire length of a chromosome's arms (**Figure 2.19c**). The cohesin also acts on the chromosome arms of

homologs at the chiasmata—the crossover points between homologous chromosomes—tethering two homologs together at their ends.



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2.19 Cohesin controls the separation of chromatids and chromosomes in mitosis and meiosis. (a) Structure of a cohesin ring around a segment of a pair of chromatids. (b) In mitosis, cohesin keeps sister chromatids together until anaphase, when its breakdown allows chromatids to separate. (c) In meiosis, cohesin along the chromosome arms breaks down in anaphase I, but cohesin remains at the centromere, keeping sister chromatids together until anaphase II. (In parts b and c, the cohesin ring structure is simplified.)

Description

In a: The diagram of cohesion shows two horizontal sister chromatids inside a circular loop labeled as cohesion ring having a bunch of circular structures hanging at the bottom. At the top end of the ring, two circular discs appear and an opening is present between them.

In b: The first diagram shows two rod shaped sister chromatids during metaphase. The sister kinetochores are drawn as dots on the middle of the chromatids and originate from a grey structure at the center of the chromatids. The spindle fibers emerge out from these grey structures. The cohesion, represented by pink circles in between the chromatids, holds the sister chromatids together. A caption reads, “Sister kinetochores orient toward different poles, and cohesin keeps sister chromatids together.” This leads to next stage metaphase where breakdown of cohesin occurs. The breakdown in cohesin is shown by the pink circles breaking in half. As a result, the sister chromatids separate. A caption reads, “The breakdown of cohesin allows sister chromatids to separate.” The chromatids separate as lateral V shaped with their open ends close and tips farther.

In c: The first diagram shows 2 V shaped chromosomes together during metaphase 1. Cohesin, represented by pink circles, are located along the chromosome arms. Cohesin also holds the homologs together at the chiasmata. A blue tiny dot is present in the cohesin structure in two molecules above and two below the center and one of them is labeled as shugoshin. The spindle fibers are emerging from green structures present at around center of the chromosomes. A caption reads, “Cohesin along chromosome arms holds homologs together at chiasmata.” Breakdown of cohesin along arms occurs and this leads to next stage anaphase 1.

In the second diagram, the breakdown in cohesin is shown by the pink circles breaking in half. With the breakdown of cohesin, the almost V shaped chromosome homologs separate from each other and migrate to opposite sides. The spindle fibers emerge out from green structures. A caption reads, “Cohesin along chromosome arms breaks down, allowing homologs to separate but cohesin at the centromere is protected by shugoshin.” Breakdown of centromeric cohesion occurs and this leads to next stage anaphase 2. The third diagram shows anaphase 2. The shugoshin is degrade as the blue dots have disappeared. The spindle fibers emerge out from green structures and almost V shaped chromatids are pulled away from each other. A caption reads, “Shugoshin is degraded. Cohesin at the centromeres breaks down, allowing chromatids to separate.”

In anaphase I, cohesin along the chromosome arms is broken, allowing the two homologs to separate. However, cohesin at the centromere is protected by a protein called shugoshin, which means

"guardian spirit" in Japanese. Because of the protective action of shugoshin, the centromeric cohesin remains intact and prevents the separation of the two sister chromatids during anaphase I. Shugoshin is subsequently degraded. At the end of metaphase II, the centromeric cohesin—no longer protected by shugoshin—breaks down, allowing the sister chromatids to separate in anaphase II, just as they do in mitosis (see Figure 2.19c). ❯ **TRY PROBLEM 34**

THINK-PAIR-SHARE Question 8

CONCEPTS

Cohesin holds sister chromatids together during the early part of mitosis. In anaphase, cohesin breaks down, allowing sister chromatids to separate. In meiosis, cohesin is protected at the centromeres during anaphase I, so homologous chromosomes, but not sister chromatids, separate in meiosis I. The breakdown of centromeric cohesin allows sister chromatids to separate in anaphase II of meiosis.

✔ CONCEPT CHECK 6

How does shugoshin affect sister chromatids in meiosis I and meiosis II?

Meiosis in the Life Cycles of Animals and Plants

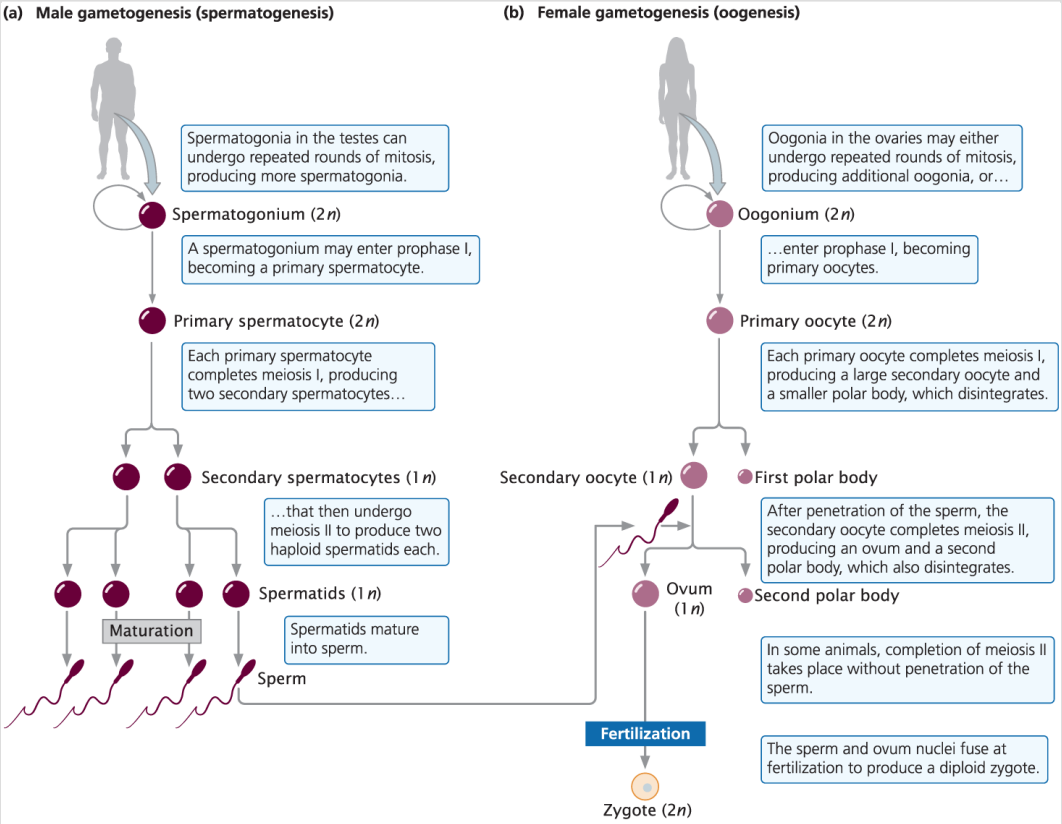
The overall result of meiosis is four haploid cells that are genetically variable. Let's see where meiosis fits into the life cycles of a multicellular animal and a multicellular plant.

MEIOSIS IN ANIMALS

The production of gametes in a male animal, a process called **spermatogenesis**, takes place in the testes. There, diploid primordial germ cells divide mitotically to produce diploid cells called **spermatogonia** (**Figure 2.20a**). Each spermatogonium can undergo repeated rounds of mitosis, giving rise to numerous additional spermatogonia. Alternatively, a spermatogonium can initiate meiosis and enter prophase I. Now called a **primary spermatocyte**, the cell is still diploid because the homologous chromosomes have not yet separated. Each primary spermatocyte completes meiosis I, giving rise to two haploid **secondary spermatocytes** that then undergo meiosis II, with each producing two haploid **spermatids**. Thus, each primary spermatocyte produces a total of four haploid spermatids, which mature and develop into sperm.

The production of gametes in a female animal, a process called **oogenesis**, begins much as spermatogenesis does. Within the ovaries, diploid primordial germ cells divide mitotically to produce **oogonia** (**Figure 2.20b**). Like spermatogonia, oogonia can undergo repeated rounds of mitosis or they can enter meiosis. When they

enter prophase I, these still-diploid cells are called primary oocytes. Each primary oocyte completes meiosis I and divides.



2.20 Gamete formation in animals.

Description

The first flow chart “a” is on the left and shows the outline of a man at the top. All of the cells responsible for forming the sperm are depicted as circles. At the bottom of the flow chart are the outlines of sperm. Male gametogenesis or spermatogenesis is described in the flow chart as follows: Spermatogonium are 2 n. They may also change back to spermatogonium. A caption reads, spermatogonia in the testes can undergo repeated rounds of mitosis, producing more spermatogonia. Spermatogonium then changes to primary spermocyte. A caption reads, a spermatogonium may enter prophase 1, becoming a primary spermatocyte. Primary spermocyte further changes to 2 secondary spermocytes (1 n). A caption reads, each primary spermatocyte completes meiosis 1, producing two secondary spermatocytes. Each secondary spermocyte then leads to the

formation of 2 spermatids (1 n). A total of 4 spermatids is formed. A caption reads, that then undergo meiosis 2 to produce two haploid spermatids each. Each spermatid matures into a sperm and a total of 4 sperms are produced. A caption reads, spermatids mature into sperm.

The second flow chart “b” on the right shows the outline of a woman. The flow chart describes female gametogenesis or oogenesis as follows: Oogonium is 2 n and may also change back to oogonium. A caption reads, oogonia in the ovaries may either undergo repeated rounds of mitosis, producing additional oogonia. Oogonium then changes to Primary oocyte (2 n). A caption reads, enter prophase 1, becoming primary oocytes. The primary oocyte then leads to formation of a larger secondary oocyte (1 n) and a smaller first polar body. A caption reads, Each primary oocyte completes meiosis1, producing a large secondary oocyte and a smaller polar body, which disintegrates. A sperm from male interacts with the secondary oocyte formed and then a larger ovum (1 n) and a smaller second polar body. A caption reads, after penetration of the sperm, the secondary oocyte completes meiosis 2, producing an ovum and a second polar body, which also disintegrates. Fertilization occurs and a zygote (2 n) is produced. A caption reads, the sperm and ovum nuclei fuse at fertilization to produce a diploid zygote. In some animals, completion of meiosis 2 takes place without penetration of the sperm.

At this point, oogenesis begins to differ from spermatogenesis. In oogenesis, cytokinesis is unequal: most of the cytoplasm is allocated to one of the two haploid cells, the **secondary oocyte**. The smaller cell, which contains half of the chromosomes but only a small part of the cytoplasm, is called the **first polar body**; it may or may not divide further. The secondary oocyte completes meiosis II, and again, cytokinesis is unequal—most of the cytoplasm passes into one of the cells. The larger cell, which acquires most of the cytoplasm, is the **ovum**, the mature female gamete. The smaller cell is the **second polar body**. Only the ovum is capable of being fertilized, and the

polar bodies usually disintegrate. Oogenesis, then, produces a single mature gamete from each primary oocyte.

In mammals, oogenesis differs from spermatogenesis in another way. The formation of sperm takes place continuously in a male throughout his adult reproductive life. The formation of female gametes, however, is often a discontinuous process and may take place over a period of years. Oogenesis begins before birth; at this time, oogonia initiate meiosis and give rise to primary oocytes. Meiosis is then arrested, stalled in prophase I. Thus, a female is born with primary oocytes arrested in prophase I. In humans, this period of suspended animation may last 30 or 40 years, until rising hormone levels stimulate one or more of the primary oocytes to recommence meiosis. The first division of meiosis is completed, and a secondary oocyte is ovulated from the ovary (a process called ovulation). In humans and many other species, the second division of meiosis is then delayed until contact with the sperm. When a sperm penetrates the outer layer of the secondary oocyte, the second meiotic division takes place, the second polar body is extruded from the ovum, and the nuclei of the sperm and newly formed ovum fuse, giving rise to the zygote.

CONCEPTS

In the testes, a diploid spermatogonium undergoes meiosis, producing a total of four haploid sperm cells. In the ovary, a diploid oogonium undergoes meiosis to produce a single large ovum and smaller polar bodies that normally disintegrate.

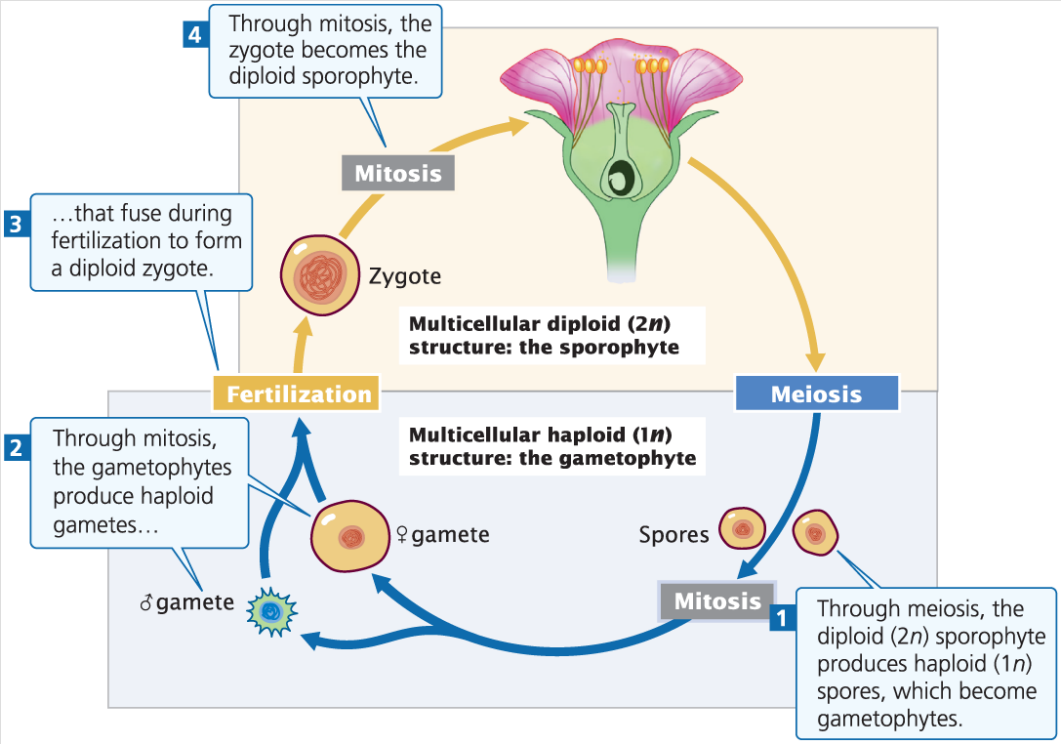
✔ CONCEPT CHECK 7

A secondary spermatocyte has 12 chromosomes. How many chromosomes will be found in the primary spermatocyte that gave rise to it?

a. 6
b. 12
c. 18
d. 24

MEIOSIS IN PLANTS

Most multicellular plants and algae have a complex life cycle that includes two distinct structures (generations): a multicellular diploid sporophyte and a multicellular haploid gametophyte. These two generations alternate; the sporophyte produces haploid spores through meiosis, and the gametophyte produces haploid gametes through mitosis (Figure 2.21). This type of life cycle is sometimes called alternation of generations. In this cycle, the immediate products of meiosis are called spores, not gametes; the spores undergo one or more mitotic divisions to produce gametes. Although the terms used to describe the plant life cycle are somewhat different from those commonly used for animals (and from some of those employed so far in this chapter), the processes in plants and animals are basically the same: in both, meiosis leads to a reduction in chromosome number, producing haploid cells.



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2.21 Plants alternate between diploid and haploid life stages. (Female, ♀; male, ♂).

Description

The upper half of the flowchart is labeled “multicellular diploid (2 n) structure: the sporophyte”, while the lower half is labeled “multicellular haploid (1 n) structure: the gametophyte”. The flowchart starts with a flower having stamen and carpel in the sporophyte stage. Meiosis occurs and two circular shaped spores are formed in the gametophyte stage. A caption reads, “Through meiosis, the diploid (2 n) sporophyte produces haploid (1 n) spores, which become gametophytes.” Mitosis occurs and a larger, circular female gamete and a smaller, circular spiky male gamete are formed in the gametophyte stage. A caption reads, “

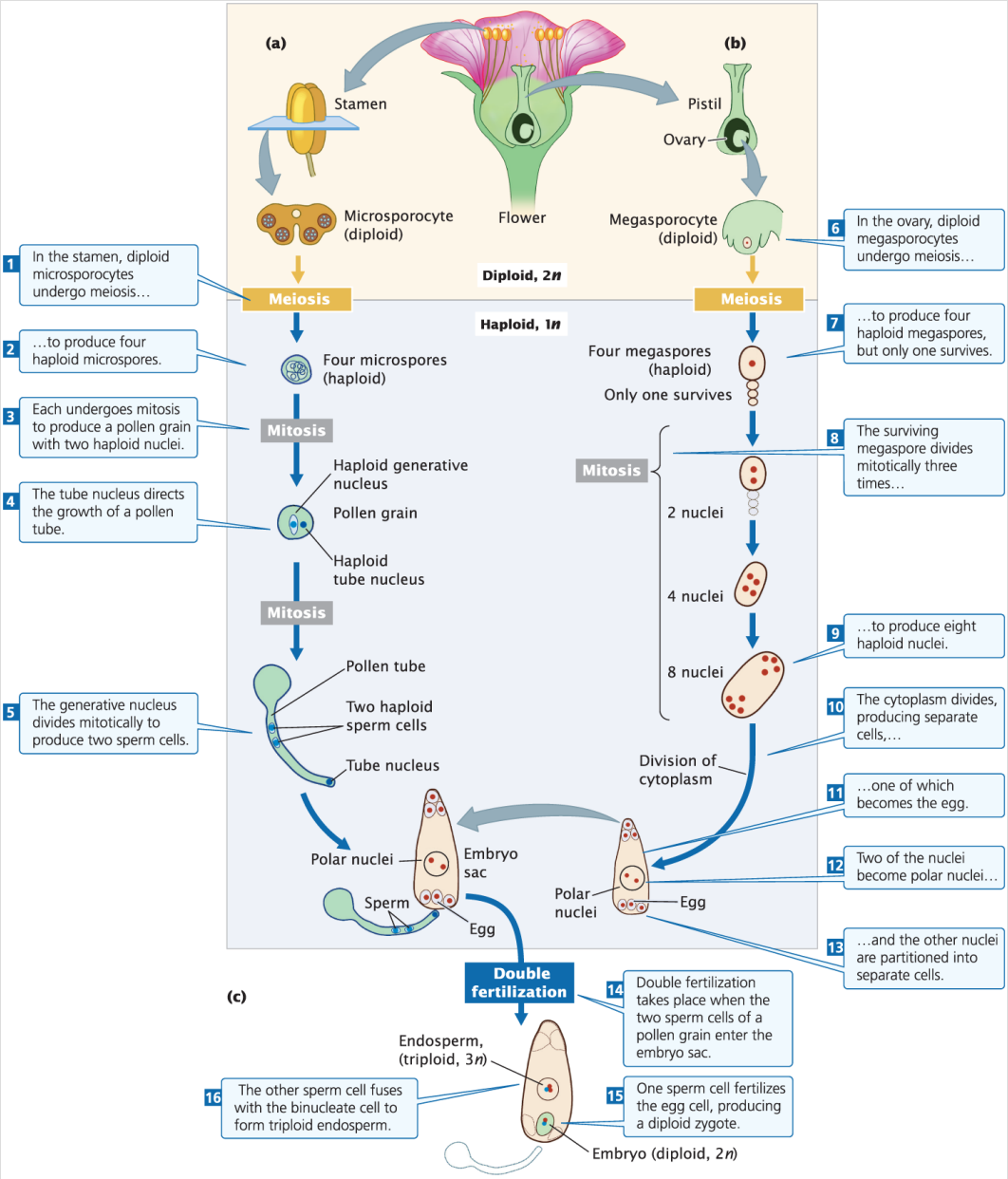
Through mitosis, the gametophytes produce haploid gametes that fuse during fertilization to form a diploid zygote.” Both these gametes undergo fertilization. . A large circular zygote is formed in the sporophyte stage. Mitosis occurs and this again leads to the same flower. A caption reads, “Through mitosis, the zygote becomes the diploid sporophyte.”

In flowering plants, the sporophyte is the obvious, vegetative part of the plant; the gametophyte consists of only a few haploid cells within the sporophyte. The flower, which is part of the sporophyte, contains the reproductive structures. In some species, both male and female reproductive structures are found in the same flower; in other species, they exist in different flowers. In either case, the male part of the flower, the stamen, contains diploid reproductive cells called **microsporocytes**, each of which undergoes meiosis to produce four haploid **microspores** (**Figure 2.22a**). Each microspore divides mitotically, producing an immature pollen grain consisting of two haploid nuclei. One of these nuclei, called the tube nucleus, directs the growth of a pollen tube. The other, termed the generative nucleus, divides mitotically to produce two sperm cells. The pollen grain, with its two haploid nuclei, is the male gametophyte.

The female part of the flower, the ovary, contains diploid cells called **megasporocytes**, each of which undergoes meiosis to produce four haploid **megaspores** (**Figure 2.22b**), only one of which survives. The nucleus of the surviving megaspore divides mitotically three times, producing a total of eight haploid nuclei that make up the female gametophyte, otherwise known as the embryo sac. Division of the cytoplasm then produces separate cells, one of which becomes the egg.

When the plant flowers, the stamens open and release pollen grains. Pollen lands on a flower's stigma—a sticky platform that sits on top of a long stalk called the style. At the base of the style is the ovary. If

a pollen grain germinates, it grows a tube down the style into the ovary. The two sperm cells pass down this tube and enter the embryo sac (**Figure 2.22c**). One of the sperm cells fertilizes the egg cell, producing a diploid zygote, which develops into an embryo. The other sperm cell fuses with two nuclei enclosed in a single cell, giving rise to a 3n (triploid) endosperm, which stores food that will be used later by the embryonic plant. These two fertilization events are collectively termed double fertilization.



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2.22 Sexual reproduction in flowering plants.

Description

At the top center of the illustration is a flower. Both the flowcharts arise from this flower. On the left side is male reproduction labeled as “a” and on the right is female reproduction labeled as “b”. The flowchart “a” starts at the top and progresses downward and arrows are drawn between each stage. Diploid microsporocytes in the stamen undergo meiosis and produce four haploid microspores. A caption reads, “In the stamen, diploid

microsporocytes undergo meiosis to produce four haploid microspores." These microspores undergo mitosis to produce a circular pollen grain with two nuclei: one is a tiny round shaped labeled as haploid

tube nucleus and other is almost rod shaped labeled as haploid generative nucleus. A caption reads, "Each undergoes mitosis to produce a pollen grain with two haploid nuclei. The tube nucleus directs

the growth of a pollen tube." Mitosis occurs and a pollen tube is produced. This pollen tube has a bulge at one end and a long tube. Two haploid sperm cells are marked as bands in its tube part. The end of pollen tube is labeled as tube nucleus. A caption reads, "The generative nucleus divides mitotically to produce two sperm cells." The flowchart "b" shows female reproduction. The ovary in pistil, a diploid megasporocyte, undergoes meiosis to produce 4 haploid megaspores where 1 is a large oval shaped and other three are tiny oval shaped. Out of these 4, only 1 survives. A caption reads, "In the ovary, diploid megasporocytes undergo meiosis to produce four haploid megaspores, but only one survives." The large surviving megaspore undergoes mitosis three times such that after first it has 2 tiny circular nuclei, after second mitosis it has 4 nuclei, and after third mitosis it has 8 nuclei. The megaspore with 8 nuclei has four clustered on each end. A caption reads, "The surviving megaspore divides mitotically three times to produce eight haploid nuclei." The cytoplasm divides. A large spore is produced with three cells arranged at one end, circular polar nuclei with 2 nuclei, and three cells at bottom where one cell in the middle is egg. A caption reads, "The cytoplasm divides, producing separate cells one of which becomes the egg. Two of the nuclei become polar nuclei and the other nuclei are partitioned into separate cells." This spore with egg from flowchart "b" and the pollen tube from male from flowchart "a" interacts. The outer covering of spore is labeled as embryo sac. Double fertilization occurs and part "c" at the bottom shows formation of a spore with an endosperm, (triploid, 3 n) and an embryo (diploid, 2 n). A caption reads, "double fertilization takes place when the two sperm cells of a pollen grain enter the embryo sac. One sperm cell fertilizes the egg cell, producing a diploid zygote. The other sperm cell fuses with the binucleate cell to form triploid endosperm."

We have now examined the place of meiosis in the sexual cycles of two organisms: a typical multicellular animal and a flowering plant. These cycles are just two of the many variations found among

eukaryotic organisms. Although the cellular processes that produce reproductive cells in plants and animals differ in the number of cell divisions, the number of haploid gametes produced, and the relative sizes of the final products, the overall result is the same: meiosis gives rise to haploid, genetically variable cells that fuse during fertilization to produce diploid progeny. **› TRY PROBLEMS 40 AND 42**

CONCEPTS

In the stamen of a flowering plant, meiosis produces haploid microspores that divide mitotically to produce haploid sperm in a pollen grain. Within the ovary, meiosis produces four haploid megaspores, only one of which divides mitotically three times to produce the eight haploid nuclei that make up the embryo sac. After pollination, one sperm fertilizes the egg cell, producing a diploid zygote; the other fuses with two nuclei to form the endosperm.

✔ CONCEPT CHECK 8

Which of the following structures is diploid?

a. Microspore
b. Egg
c. Megaspore
d. Microsporocyte

Chapter 2 Review

CHAPTER SUMMARY

Section 2.1 Learning Objective: Be able to list genetic differences between eukaryotic cells and prokaryotic cells.

- A prokaryotic cell possesses a simple structure, with no nuclear membrane and usually a single circular chromosome. A eukaryotic cell possesses a more complex structure, with a nucleus and multiple linear chromosomes consisting of DNA complexed with histone proteins.

Section 2.2 Learning Objective: Be able to compare cell division in bacteria and eukaryotes.

- Cell reproduction requires the copying of genetic material, separation of the copies, and cell division.
- In a prokaryotic cell, the single chromosome replicates, the two copies move toward opposite sides of the cell, and the cell divides. In eukaryotic cells, reproduction is more complex than in prokaryotic cells, requiring mitosis to ensure that a complete set of genetic information is transferred to each new cell, or meiosis to generate gametes that contain a copy of each chromosome.

- In eukaryotic cells, chromosomes are typically found in homologous pairs. Each functional chromosome consists of a centromere, telomeres, and multiple origins of replication. After a chromosome has been copied, the two copies remain attached at the centromere, forming sister chromatids.
- The cell cycle consists of the stages through which a eukaryotic cell passes between cell divisions. It consists of (1) interphase, in which the cell grows and prepares for division, and (2) the M phase, in which nuclear and cell division take place. The M phase consists of (1) mitosis, the process of nuclear division, and (2) cytokinesis, the division of the cytoplasm.
- Progression through the cell cycle is regulated at checkpoints that allow or prohibit the cell's progression to the next stage.
- Mitosis usually results in the production of two genetically identical cells.

Section 2.3 Learning Objective: Be able to explain how the process of meiosis produces genetic variation during sexual reproduction.

- Sexual reproduction produces genetically variable progeny. It includes meiosis, in which haploid sex cells are produced, and fertilization, the fusion of sex cells. Meiosis includes two cell divisions. In meiosis I, crossing over takes place, and homologous chromosomes separate. In meiosis II, sister chromatids separate.
- The usual result of meiosis is the production of four haploid cells that are genetically variable. Genetic variation in meiosis

is produced by crossing over and by the random distribution of maternal and paternal chromosomes.

- Cohesin holds sister chromatids together. In metaphase of mitosis and in metaphase II of meiosis, the breakdown of cohesin allows sister chromatids to separate. In meiosis I, centromeric cohesin remains intact and keeps sister chromatids together so that homologous chromosomes, but not sister chromatids, separate in anaphase I.
- In animals, a diploid spermatogonium undergoes meiosis to produce four haploid sperm cells. A diploid oogonium undergoes meiosis to produce one large haploid ovum and one or more smaller polar bodies.
- In plants, a diploid microsporocyte in the stamen undergoes meiosis to produce four pollen grains, each with two haploid sperm cells. In the ovary, a diploid megasporocyte undergoes meiosis to produce four haploid megaspores. One of these megaspores divides mitotically three times to produce eight haploid nuclei, one of which forms the egg cell. During pollination, one sperm fertilizes the egg cell and the other fuses with two haploid nuclei to form a triploid endosperm.

WORKED PROBLEMS

Problem 1

A student examines a thin section of an onion-root tip and records the number of cells that are in each stage of the cell cycle. She

observes 94 cells in interphase, 14 cells in prophase, 3 cells in prometaphase, 3 cells in metaphase, 5 cells in anaphase, and 1 cell in telophase. If the complete cell cycle in an onion-root tip requires 22 hours, what is the average duration of each stage in the cycle? Assume that all cells are in the active cell cycle (not G_0).

» Solution Strategy

What information is required in your answer to the problem?

The average duration of each stage of the cell cycle.

What information is provided to solve the problem?

- The numbers of cells in different stages of the cell cycle.
- A complete cell cycle requires 22 hours.

For help with this problem, review:

The Cell Cycle and Mitosis in Section 2.2.

» Solution Steps

This problem is solved in two steps. First, we calculate the proportions of cells in each stage of the cell cycle, which correspond to the amount of time that an average cell spends in each stage. For example, if cells spend 90% of their time in

interphase, then at any given moment, 90% of the cells will be in interphase. The second step is to convert the proportions into lengths of time, which is done by multiplying the proportions by the total time of the cell cycle (22 hours).

The proportion of cells at each stage is equal to the number of cells found in that stage divided by the total number of cells examined:

Interphase	$^{94}/_{120} = 0.783$
Prophase	$^{14}/_{120} = 0.117$
Prometaphase	$^{3}/_{120} = 0.025$
Metaphase	$^{3}/_{120} = 0.025$
Anaphase	$^{5}/_{120} = 0.042$
Telophase	$^{1}/_{120} = 0.008$

Hint: The total of all the proportions should equal 1.0.

To determine the average duration of each stage, multiply the proportion of cells in each stage by the time required for the entire cell cycle:

Interphase	$0.783 \times 22 \text{ hours} = 17.23 \text{ hours}$
Prophase	$0.117 \times 22 \text{ hours} = 2.57 \text{ hours}$
Prometaphase	$0.025 \times 22 \text{ hours} = 0.55 \text{ hour}$
Metaphase	$0.025 \times 22 \text{ hours} = 0.55 \text{ hour}$
Anaphase	$0.042 \times 22 \text{ hours} = 0.92 \text{ hour}$
Telophase	$0.008 \times 22 \text{ hours} = 0.18 \text{ hour}$

Hint: The total time for all stages should equal 22 hours.

Problem 2

A cell in G_1 of interphase has 8 chromosomes. How many chromosomes and how many DNA molecules will be found per cell as this cell progresses through the following stages: G_2, metaphase of mitosis, anaphase of mitosis, after cytokinesis following mitosis, metaphase I of meiosis, metaphase II of meiosis, and after cytokinesis following meiosis II?

» Solution Strategy

What information is required in your answer to the problem?

The number of chromosomes and the number of DNA molecules present per cell at different stages of the cell cycle and meiosis.

What information is provided to solve the problem?

The number of chromosomes in the cell in G_1.

For help with this problem, review:

Connecting Concepts: Counting Chromosomes and DNA Molecules in Section 2.2.

» Solution Steps

Remember the rules about counting chromosomes and DNA molecules: (1) to determine the number of chromosomes, count the functional centromeres; (2) to determine the number of DNA molecules, determine whether sister chromatids exist. If sister chromatids are present, the number of DNA molecules is two times the number of chromosomes. If the chromosomes are unreplicated (don't contain sister chromatids), the number of DNA molecules equals the number of chromosomes. Think carefully about when and how the numbers of chromosomes and DNA molecules change in the course of mitosis and meiosis.

Hint: These two rules are important for answering the question.

The number of DNA molecules increases only in the S phase, when DNA replicates; the number of DNA molecules decreases only when the cell divides. Chromosome number increases only when sister chromatids separate in anaphase of mitosis and in anaphase II of meiosis (homologous chromosomes, not chromatids, separate in anaphase I of meiosis). Like the number of DNA molecules, chromosome number is reduced only by cell division.

Recall: Chromosome number increases only when chromatids separate. The number of DNA molecules increases only in the S phase.

Let's now apply these principles to the problem. A cell in G_1 has 8 chromosomes, and sister chromatids are not present, so 8 DNA molecules are present per cell in G_1. DNA replicates in the S phase, and now each chromosome consists of two chromatids, so in G_2, $2 \times 8 = 16$ DNA molecules are present per cell. However, the two copies of each DNA molecule remain attached at the centromere, so there are still only 8 chromosomes present. As the cell passes through prophase and metaphase of the cell cycle, the number of chromosomes and the number of DNA molecules remain the same, so at metaphase, there are 16 DNA molecules and 8 chromosomes. In anaphase, the sister chromatids separate, and each becomes an independent chromosome; at this point, the number of chromosomes increases from 8 to 16. This increase is temporary, lasting only until the cell divides in telophase or subsequent to it. The number of DNA molecules remains at 16 in anaphase. The

number of DNA molecules and chromosomes per cell is reduced by cytokinesis after telophase because the 16 chromosomes and DNA molecules are now distributed between two cells. Therefore, after cytokinesis, each cell has 8 DNA molecules and 8 chromosomes, the same numbers that were present at the beginning of the cell cycle.

Now, let's trace the numbers of DNA molecules and chromosomes through meiosis. At G_1, there are 8 chromosomes and 8 DNA molecules. The number of DNA molecules increases to 16 in the S phase, but the number of chromosomes remains at 8 (each chromosome has two chromatids). The cell therefore enters metaphase I having 16 DNA molecules and 8 chromosomes. In anaphase I of meiosis, homologous chromosomes separate, but the number of chromosomes remains at 8. After cytokinesis, the original 8 chromosomes are distributed between two cells, so the number of chromosomes per cell falls to 4 (each with two chromatids). The original 16 DNA molecules are also distributed between two cells, so the number of DNA molecules per cell is 8. There is no DNA synthesis in interkinesis, and each cell still maintains 4 chromosomes and 8 DNA molecules through metaphase II. In anaphase II, the two sister chromatids of each chromosome separate, temporarily raising the number of chromosomes per cell to 8, whereas the number of DNA molecules per cell remains at 8. After cytokinesis, the chromosomes and DNA molecules are again distributed between two cells, providing 4 chromosomes and 4 DNA molecules per cell. These results are summarized in the following table:

Stage	Number of chromosomes per cell	Number of DNA molecules per cell
G_1	8	8
G_2	8	16
Metaphase of mitosis	8	16
Anaphase of mitosis	16	16
After cytokinesis following mitosis	8	8
Metaphase I of meiosis	8	16
Metaphase II of meiosis	4	8
After cytokinesis following meiosis II	4	4

IMPORTANT TERMS

prokaryote
eukaryote
bacteria
archaea
nucleus
histone
chromatin
virus
homologous pair
diploid
haploid
polyploid
centromere
telomere
origin of replication
sister chromatid
cell cycle
checkpoint
interphase
M (mitotic) phase
mitosis
cytokinesis
prophase
condensin
prometaphase

metaphase
anaphase
telophase
meiosis
fertilization
prophase I
synapsis
bivalent
tetrad
crossing over
metaphase I
anaphase I
telophase I
interkinesis
prophase II
metaphase II
anaphase II
telophase II
recombination
cohesin
spermatogenesis
spermatogonium
primary spermatocyte
secondary spermatocyte
spermatid
oogenesis
oogonium
primary oocyte

secondary oocyte
first polar body
ovum
second polar body
microsporocyte
microspore
megasporocyte
megaspore

ANSWERS TO CONCEPT CHECKS

1. Bacteria and archaea are prokaryotes. They differ from eukaryotes in possessing no nucleus, a genome that usually consists of a single circular chromosome, and a smaller amount of DNA.
2. b
3. The kinetochore is the point at which spindle microtubules attach to the chromosome during cell division. If the kinetochore were missing, spindle microtubules would not attach to the chromosome, the chromosome would not be drawn into a newly formed nucleus, and the resulting daughter cells would be missing a chromosome.
4. a
5. c
6. During anaphase I, shugoshin protects cohesin at the centromere from the action of separase, so centromeric cohesin remains intact, and the sister chromatids remain

together. Subsequently, shugoshin breaks down, so centromeric cohesin is cleaved by separase in anaphase II, and the sister chromatids separate.

7. d
8. d

Chapter 2 Assessment

COMPREHENSION QUESTIONS

Section 2.1

1. What are some genetic differences between prokaryotic and eukaryotic cells?
2. Why are viruses often used in the study of genetics?

Section 2.2

3. List three fundamental events that must take place in cell reproduction.
4. Outline the process by which prokaryotic cells reproduce.
5. Name three essential structural elements of a functional eukaryotic chromosome, and describe their functions.
6. Sketch and identify four different types of chromosomes based on the position of the centromere.
7. List the stages of interphase and the major events that take place in each stage.
8. What are checkpoints? List some of the important checkpoints in the cell cycle.
9. List the stages of mitosis and the major events that take place in each stage.
10. Briefly describe how the chromosomes move toward the spindle poles during anaphase.
11. What are the genetically important results of the cell cycle and mitosis?
12. Why are the two cells produced by the cell cycle genetically identical?

Section 2.3

13. What are the stages of meiosis, and what major events take place in each stage?
14. What are the major results of meiosis?
15. What two processes unique to meiosis are responsible for genetic variation? At what point in meiosis do these processes take place?
16. How does anaphase I of meiosis differ from anaphase of mitosis?
17. Briefly explain why sister chromatids remain together in anaphase I but separate in anaphase II of meiosis.
18. Outline the processes of spermatogenesis and oogenesis in animals.
19. Outline the processes of male gamete formation and female gamete formation in plants.

APPLICATION QUESTIONS AND PROBLEMS

Introduction

20. *Answer the following questions about the blind men's riddle, presented in the introduction to this chapter.
    a. What do the two socks of a pair represent in the cell cycle?
    b. In the riddle, each blind man buys his own pairs of socks, but the clerk places all the pairs in one bag. Thus, there are two pairs of socks of each color in the bag (two black pairs, two blue pairs, two gray pairs, etc.). What do the two pairs (four socks in all) of each color represent?
    c. What is the thread that connects the two socks of a pair?
    d. What is the molecular knife that cuts the thread holding the two socks of a pair together?
    e. What in the riddle performs the same function as spindle microtubules?
    f. What would happen if one man failed to grasp his sock of a particular pair? How does that outcome relate to events in the cell cycle?

Section 2.1

21. A cell has a circular chromosome and no nuclear membrane. Its DNA is complexed with some histone proteins. Does this cell belong to a bacterium, an archaean, or a eukaryote? Explain your reasoning.

Section 2.2

22. A certain species has three pairs of chromosomes: an acrocentric pair, a metacentric pair, and a submetacentric pair. Draw a cell of this species as it would appear in metaphase of mitosis.

23. Examine **Figure 2.6a**. What type of chromosome (metacentric, submetacentric, acrocentric, or telocentric) is chromosome 1? What about chromosome 4?

24. *A biologist examines a series of cells and counts 160 cells in interphase, 20 cells in prophase, 6 cells in prometaphase, 2 cells in metaphase, 7 cells in anaphase, and 5 cells in telophase. If the complete cell cycle requires 24 hours, what is the average duration of the M phase in these cells? Of metaphase?

25. In what stage of mitosis is the cell illustrated in the chapter opening figure (p. 17)?

Section 2.3

26.

What role would mitosis and/or meiosis play in human reproduction under the theory of preformationism (see Chapter 1)?

27. A certain species has three pairs of chromosomes: one acrocentric pair and two metacentric pairs. Draw a cell of this species as it would appear in the following stages of meiosis:
    a. Metaphase I
    b. Anaphase I
    c. Metaphase II
    d. Anaphase II

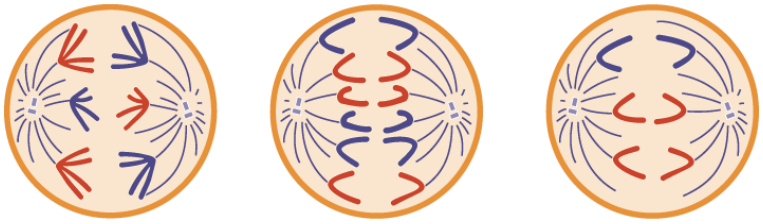
28. Construct a table similar to that in **Figure 2.12** for the different stages of meiosis, giving the number of chromosomes per cell and the number of DNA molecules

per cell for a cell that begins with 4 chromosomes (two homologous pairs) in G_1. Include the following stages in your table: G_1, S, G_2, prophase I, metaphase I, anaphase I, telophase I (after cytokinesis), prophase II, metaphase II, anaphase II, and telophase II (after cytokinesis).

29. *A cell in G_1 of interphase has 12 chromosomes ($2n = 12$). How many chromosomes and DNA molecules will be found per cell when this original cell progresses to the following stages?
    a. G_2 of interphase
    b. Metaphase I of meiosis
    c. Prophase of mitosis
    d. Anaphase I of meiosis
    e. Anaphase II of meiosis
    f. Prophase II of meiosis
    g. After cytokinesis following mitosis
    h. After cytokinesis following meiosis II

30. How are the events that take place in spermatogenesis and oogenesis similar? How are they different?

31. *All of the following cells, shown in various stages of mitosis and meiosis, come from the same rare species of plant.
    a. What is the diploid number of chromosomes in this plant?
    b. Give the names of each stage of mitosis or meiosis shown.
    c. Give the number of chromosomes and number of DNA molecules per cell present at each stage.



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Description
The first cell on the left shows three X-shaped chromosomes being pulled to the left and three being pulled to the right with the help of spindle fibers attached to their centromeres. On the left side, the top and bottom

chromosomes are red and the middle one is blue while on the right, top and bottom are blue and middle one is red.

The second cell in the center shows six lateral V-shaped sister chromatids pulled to the left and six pulled to the right. On both the sides, blue chromatids are at first, fourth, and fifth positions while red chromatids are at second, third and sixth positions.

The third cell on the right has three chromatids being pulled to the left and three chromatids are being pulled to the right. On both the sides, the blue chromatids are at the top and rest are red.

32. *The amount of DNA per cell of a particular species is measured in cells found at various stages of meiosis, and the following amounts are obtained:

Amount of DNA per cell in picograms (pg)		
_____ 3.7 pg	_____ 7.3 pg	_____ 14.6 pg

Match the amounts of DNA above with the corresponding stages of meiosis (*a* through *f*, below). You may use more than one stage for each amount of DNA.

Stage of meiosis

a. G_1
b. Prophase I
c. G_2
d. Following telophase II and cytokinesis
e. Anaphase I
f. Metaphase II

33. CONNECT Tawa has oculocutaneous type 2 albinism (see introduction to Chapter 1). How many copies of the DNA sequence that causes albinism will be present in Tawa's cells during the following stages?
    a. Anaphase of mitosis
    b. G_1 of interphase
    c. Prophase of mitosis
    d. Anaphase II of meiosis
    e. Metaphase I of meiosis

f. After cytokinesis following meiosis II
g. After cytokinesis following mitosis

34. *How would each of the following events affect the outcome of mitosis or meiosis?
    a. Mitotic cohesin fails to form early in mitosis.
    b. Shugoshin is absent during meiosis.
    c. Shugoshin does not break down after anaphase I of meiosis.
    d. Separase is defective.

35. *A cell in prophase II of meiosis contains 12 chromosomes. How many chromosomes would be present in a cell from the same organism if it were in prophase of mitosis? Prophase I of meiosis?

36. A cell has 8 chromosomes in G_1 of interphase. Draw a picture of this cell with its chromosomes at the following stages. Indicate how many DNA molecules are present at each stage.
    a. Metaphase of mitosis
    b. Anaphase of mitosis
    c. Anaphase II of meiosis
    d. Diplotene of meiosis I

37. *The fruit fly *Drosophila melanogaster* (left) has four pairs of chromosomes, whereas the house fly *Musca domestica* (right) has six pairs of chromosomes. In which species would you expect to see more genetic variation among the progeny of a cross? Explain your answer.

Left: © Graphic Science/Alamy Stock Photo; right: © Debug/ iStock Photo.

38. *A cell has two pairs of submetacentric chromosomes, which we will call chromosomes I_a, I_b, II_a, and II_b (chromosomes I_a and I_b are homologs, and

chromosomes II_a and II_b are homologs). Allele *M* is located on the long arm of chromosome I_a, and allele *m* is located at the same position on chromosome I_b. Allele *P* is located on the short arm of chromosome I_a, and allele *p* is located at the same position on chromosome I_b. Allele *R* is located on chromosome II_a and allele *r* is located at the same position on chromosome II_b.

a. Draw these chromosomes, identifying genes *M*, *m*, *P*, *p*, *R*, and *r* as they might appear in metaphase I of meiosis. Assume that there is no crossing over.

b. Taking into consideration the random separation of chromosomes in anaphase I, draw the chromosomes (with genes identified) present in all possible types of gametes that might result from this cell's undergoing meiosis. Assume that there is no crossing over.

39. A horse has 64 chromosomes and a donkey has 62 chromosomes. A cross between a female horse and a male donkey produces a mule, which is usually sterile. How many chromosomes does a mule have? Can you think of any reasons for the fact that most mules are sterile?

40. *Normal somatic cells of horses have 64 chromosomes ($2n = 64$). How many chromosomes and DNA molecules will be present in the following types of horse cells?

Cell type	Number of chromosomes	Number of DNA molecules
a. Spermatogonium	_____	_____
b. First polar body	_____	_____
c. Primary oocyte	_____	_____
d. Secondary spermatocyte	_____	_____

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41. Indicate whether each of the following cells is haploid or diploid.

Cell type	Haploid or diploid?
Microspore	_____
Primary spermatocyte	_____
Microsporocyte	_____
First polar body	_____
Oogonium	_____
Spermatid	_____
Megaspore	_____
Ovum	_____
Secondary oocyte	_____
Spermatogonium	_____

42. *A primary oocyte divides to give rise to a secondary oocyte and a first polar body. The secondary oocyte then divides to give rise to an ovum and a second polar body.
    a. Is the genetic information found in the first polar body identical to that found in the secondary oocyte? Explain your answer.

b. Is the genetic information found in the second polar body identical to that in the ovum? Explain your answer.

CHALLENGE QUESTIONS

Section 2.3

43. CONNECT An autotriploid (3*n*; see Chapter 8) has three sets of homologous chromosomes in each cell instead of the usual pairs of homologous chromosomes present in diploid individuals (2*n*). How would mitosis and meiosis be different in an autotriploid? (Hint: See Figure 8.26.)

44. From 80% to 90% of the most common human chromosome abnormalities arise because the chromosomes fail to divide properly in oogenesis. Can you think of a reason why failure of chromosome division might be more common in female gametogenesis than in male gametogenesis?

45. On average, what proportion of the genome in the following pairs of humans would be exactly the same if no crossing over took place? (For the purposes of this question only, we will ignore the special case of the X and Y sex chromosomes and assume that all genes are located on nonsex chromosomes.)
    a. Father and child
    b. Mother and child
    c. Two full siblings (offspring that have the same two biological parents)
    d. Half siblings (offspring that have only one biological parent in common)
    e. Uncle and niece
    f. Grandparent and grandchild

46. *Female bees are diploid, and male bees are haploid. The haploid males produce sperm and can successfully mate with diploid females. Fertilized eggs develop into females and unfertilized eggs develop into males. How do you think the process of sperm production in male bees differs from sperm production in other animals?

💬 ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 2.2

1. A chromosome consists of two sister chromatids. Does the genetic information on the two sister chromatids come from only one parent or from both parents? Explain your reasoning.

2. Are homologous pairs of chromosomes present in mitosis? Explain your reasoning.

Section 2.3

3. A species has two pairs of chromosomes: one metacentric and one submetacentric. Draw pictures of a cell of this species as it would appear in the following stages:
   a. Metaphase of mitosis
   b. Anaphase of mitosis
   c. Metaphase I of meiosis
   d. Anaphase I of meiosis
   e. Anaphase II of meiosis

4. A cell has 8 chromosomes in metaphase II of meiosis. How many chromosomes and DNA molecules will be present per cell in this same organism at the following stages?
   a. Prophase of mitosis
   b. Metaphase I of meiosis
   c. Anaphase of mitosis
   d. Anaphase II of meiosis
   e. Anaphase I of meiosis
   f. After cytokinesis following mitosis
   g. After cytokinesis following meiosis II

5. What is the difference between sister chromatids and homologous chromosomes?

6. List as many similarities and differences between mitosis and meiosis as you can. Which differences do you think are most important, and why?

7. Describe how and where each of the following terms applies to mitosis, meiosis, or both: (1) replication; (2) pairing; (3) separation.

8. Do you know of any genetic diseases or disorders that result from errors in mitosis or meiosis? How do errors in mitosis or meiosis bring about these diseases or disorders?

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Section 2.1

1. Create a concept map that compares the characteristics and relationships of archaea, bacteria, eukaryotes, and viruses. Your map should have a minimum of 12 concepts and links.

Section 2.2

2. Summarize the structures of eukaryotic chromosomes by creating a concept map that links the following terms: chromosome, origin of replication, DNA molecule, kinetochore, homologous pair, sister chromatids, telomere, and centromere.

Section 2.3

3. Create a concept map that outlines the role of cohesin in the separation of sister chromatids and homologous chromosomes in mitosis and meiosis.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 3

Basic Principles of Heredity

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Blond hair occurs in 5%–10% of dark-skinned Solomon Islanders. Research demonstrates that blond hair in this group is a recessive trait and has a different genetic basis from blond hair in Europeans.

The Genetics of Blond Hair in the South Pacific

A thousand miles northeast of Australia lies an ancient chain of volcanic and coral islands known as the Solomons (Figure 3.1). The Solomon Islands were first inhabited some 30,000 years ago, when Neanderthals still roamed northern Europe. Today, the people of the Solomons are culturally diverse but consist largely of Melanesians, a group that also inhabits other South Pacific islands. Most people from the Solomon Islands have dark skin. Remarkably, 5%–10% also have strikingly blond hair; in fact, people of the Solomon Islands have the highest frequency of blond hair outside Europe.

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3.1 Map of the Solomon Islands.

Description

The illustration shows an earth with a black square highlighting the Solomon Island. The map which is zoomed out of the illustration shows the Solomon Islands in the Solomon Sea. The Islands are to the east of Papua New Guinea. Australia is south-west to the Solomon Islands. Vanuatu and New Caledonia are south-east to the islands and South Pacific Sea is in north-east. Coral Sea is to the south of these islands, South Pacific Sea is north east to these islands and Bismarck Sea is in the north-west side.

How did the Solomon Islanders get their blond hair? A number of hypotheses have been proposed over the years. Some suggested that the blond islanders had naturally dark hair that was bleached by the sun and salt water. Others proposed that the blond hair color was caused by diet. Still others suggested that it was the result of genes for blond hair left by early European explorers.

The mystery of the blond Solomon Islanders was solved in 2012 by geneticists Eimear Kenny and Sean Myles and their colleagues. Their research demonstrated that blond hair on the islands is, in fact, caused by a gene, but not one left by Europeans—blond hair in Solomon Islanders and in Europeans has completely separate evolutionary origins.

To search for the origin of blond hair among the people of the Solomon Islands, the geneticists collected saliva and hair samples from over 1200 people on the islands, from which they extracted DNA. In a type of analysis known as a genome-wide association study, they looked for statistical associations between the presence of blond hair and thousands of genetic variants scattered across the genome. Right away, they detected a strong correlation between the

presence of blond hair and a particular genetic variant located on the short arm of chromosome 9. This region of chromosome 9 contains the tyrosinase-related protein 1 (*TYRP1*) gene, which encodes an enzyme known to play a role in the production of melanin and to affect pigmentation in mice. The researchers found a single base difference between the DNA of islanders with blond hair and that of islanders with dark hair: the blonds had a thymine (T) base instead of cytosine (C) in their *TYRP1* gene.

Further research showed that blond hair in Solomon Islanders is a recessive trait, meaning that blonds carry two copies of the blond version of the gene (*TT*)—one inherited from each parent. Dark hair is dominant: dark-haired islanders carry either one (*CT*) or two (*CC*) copies of the dark-hair version of the gene. Thus, many dark-haired islanders are heterozygous, carrying a hidden copy of the blond gene that can be passed on to their offspring. A DNA analysis of 900 Solomon Islanders demonstrated that over 40% of dark-haired islanders carry a blond gene. Interestingly, the C to T mutation in the *TYRP1* gene that causes blond hair in Solomon Islanders is rare outside the South Pacific, suggesting that the mutation arose independently within the Melanesian population. There is no evidence that the gene was inherited from Europeans.

THINK-PAIR-SHARE Question 1

The genetics of blond hair in Solomon Islanders differs from that in Europeans in other ways as well. In Europeans, variations in at least eight different genes have been associated with blond hair. In 2015, researchers examined one of these genes (called *KITLG*) and found that the mutation causing blond hair occurred not in the gene itself but in a region of DNA that affects the expression of the *KITLG* gene. The *KITLG* gene produces a protein that is involved in a number of functions, including melanocyte development and melanin synthesis.

THINK-PAIR-SHARE

- Why is knowing the genetic basis of a trait such as blond hair important? Why would scientists go to the trouble to investigate the genetic basis of blond hair in Solomon Islanders?
- If a blond-haired person from northern Europe mated with a blond Solomon Islander, what proportion of their offspring would be expected to have blond hair? Explain your reasoning.

This chapter is about the principles of heredity: how genes—such as the one for blond hair in Solomon Islanders—are passed from generation to generation and how factors such as dominance influence their inheritance. The principles of heredity were first put forth by Gregor Mendel, so we begin this chapter by examining Mendel's scientific achievements. We then turn to simple genetic crosses in which a single characteristic is examined. We consider some techniques for predicting the outcome of genetic crosses, and then turn to crosses in which two or more characteristics are

examined. We see how the principles applied to simple genetic crosses and the ratios of offspring they produce can serve as the key to understanding more complicated crosses. The chapter ends with a discussion of statistical tests for analyzing crosses.

Throughout this chapter, a number of concepts are interwoven: Mendel's principles of segregation and independent assortment, probability, and the behavior of chromosomes. These concepts might at first appear to be unrelated, but they are actually different views of the same phenomenon, because the genes that undergo segregation and independent assortment are located on chromosomes. In this chapter, we examine these different views and clarify how they are related.

3.1 Gregor Mendel Discovered the Basic Principles of Heredity

It was in the early 1900s that the principles of heredity first became widely known among biologists. Surprisingly, these principles had been discovered some 44 years earlier by an Augustinian priest named Gregor Johann Mendel (1822–1884) (Figure 3.2).

James King-Holmes/Science Source.

3.2 Gregor Johann Mendel discovered the principles of heredity by experimenting with peas.

Mendel was born in what is now part of the Czech Republic. Although his parents were simple farmers with little money, he

received a sound education and was admitted to the Augustinian monastery in Brno in September 1843. After graduating from seminary, Mendel became an ordained priest and was appointed to a teaching position in a local school. He excelled at teaching, and the abbot of the monastery recommended him for further study at the University of Vienna, which he attended from 1851 to 1853. There, Mendel enrolled in the newly opened Physics Institute and took courses in mathematics, chemistry, entomology, paleontology, botany, and plant physiology. It was probably there that Mendel acquired knowledge of the scientific method, which he later applied so successfully to his genetic experiments.

After two years of study in Vienna, Mendel returned to Brno, where he taught school and began his experimental work with pea plants. He conducted breeding experiments from 1856 to 1863 and presented his results publicly at meetings of the Brno Natural Science Society in 1865. Mendel's paper based on these lectures was published in 1866. However, in spite of widespread interest in heredity, the effect of his research on the scientific community was minimal. At the time, no one seemed to have noticed that Mendel had discovered the basic principles of inheritance.

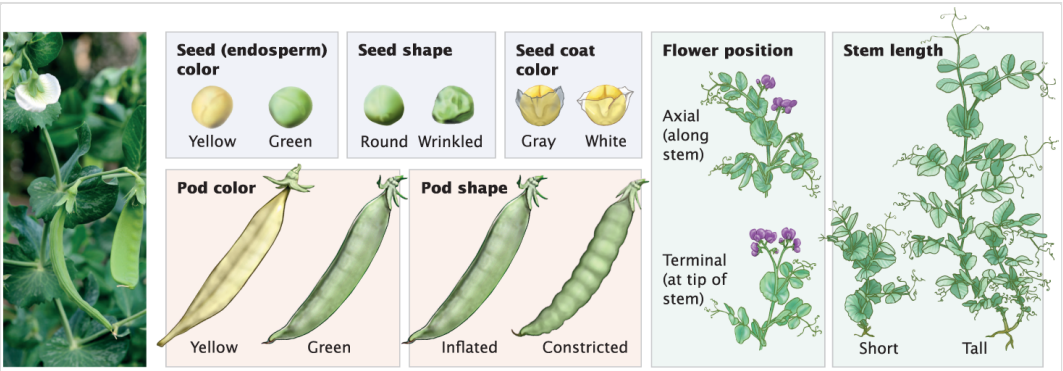
In 1868, Mendel was elected abbot of his monastery, and increasing administrative duties brought an end to his teaching and, eventually, to his genetic experiments. He died at the age of 61 on January 6, 1884, unrecognized for his contribution to genetics.

The significance of Mendel's discovery was not recognized until 1900, when three botanists—Hugo de Vries, Erich Tschermak von Seysenegg, and Carl Correns—began independently conducting similar experiments with plants and arrived at conclusions similar to those of Mendel. Coming across Mendel's paper, they interpreted their results in accord with his principles and drew attention to his pioneering work.

Mendel's Success

Mendel's approach to the study of heredity was effective for several reasons. Foremost was his choice of experimental subject, the pea plant *Pisum sativum* (**Figure 3.3**), which offered clear advantages for genetic investigation. The plant is easy to cultivate, and Mendel had the monastery garden and greenhouse at his disposal. Compared with some other plants, peas grow relatively rapidly, completing an entire generation in a single growing season. By today's standards, one generation per year seems frightfully slow—fruit flies complete a generation in 2 weeks and bacteria in 20 minutes—but Mendel was under no pressure to publish quickly and was able to follow the inheritance of individual characteristics for several generations. Had he chosen to work on an organism with a longer generation time—horses, for example—he might never have discovered the basis of inheritance. Pea plants also produce many offspring—their seeds—which allowed Mendel to detect meaningful mathematical ratios in the traits he observed in the progeny. The numerous varieties of peas

that were available to Mendel were also crucial to his success because they differed in various traits and were genetically pure. Mendel was therefore able to begin with plants of variable and known genetic makeup.



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Photograph by Charles Stirling/Alamy.

3.3 Mendel used the pea plant *Pisum sativum* in his studies of heredity. He examined seven characteristics that appeared in the seeds and in plants grown from the seeds.

Description

The illustration shows a photo of pea plant on the extreme left with a white flower. The top 3 panels demonstrate seed colors, seed shape, and seed coat color. The seed colors are yellow and green. The seed shapes are round and wrinkled and the seed coat colors are white and gray. The bottom 2 panels show pod color and shape. The pod colors are yellow and green, and the pod shapes are inflated and constricted. In inflated pod, the pod is appearing smooth from the outside. In a constricted pod the pod hugs the beans tightly so that it looks bumpy. The last 2 panels on the right show plant characteristics: flower position and stem length. The flowers may be positioned at the terminal tips of the stems or at axial points that occur all along the stem. The flowers in this panel are purple. Stem lengths are either short or tall.

Much of Mendel's success can be attributed to the seven characteristics of pea plants that he chose for study (see Figure 3.3). He avoided characteristics that display a range of variation; instead, he focused his attention on those that exist in two easily differentiated forms, such as white versus gray seed coats, round versus wrinkled seeds, and inflated versus constricted pods.

THINK-PAIR-SHARE Question 2

Finally, Mendel was successful because he adopted an experimental approach and interpreted his results by using mathematics. Unlike many earlier investigators who simply described the *results* of crosses, Mendel formulated *hypotheses* based on his initial observations and then conducted additional crosses to test his hypotheses. He kept careful records of the numbers of progeny possessing each trait and computed ratios of the different traits. He was adept at seeing patterns in detail and was patient and thorough, conducting his experiments for 10 years before attempting to write up his results. ❯ **TRY PROBLEM 13**

CONCEPTS

Gregor Mendel put forth the basic principles of inheritance, publishing his findings in 1866. Much of Mendel's success can be attributed to the seven characteristics of pea plants that he studied and his experimental approach.

✔ CONCEPT CHECK 1

Which of the following factors did not contribute to Mendel's success in his study of heredity?

a. His use of the pea plant
b. His study of plant chromosomes
c. His adoption of an experimental approach
d. His use of mathematics

Genetic Terminology

Before we examine Mendel's crosses and the conclusions that he drew from them, a review of some terms commonly used in genetics will be helpful (Table 3.1). The term *gene* is a word that Mendel never knew. It was not coined until 1909, when Danish botanist Wilhelm Johannsen first used it. The definition of *gene* varies with the context of its use, so its definition will change as we explore different aspects of heredity. For our present use in the context of genetic crosses, we define a **gene** as an inherited factor that determines a characteristic.

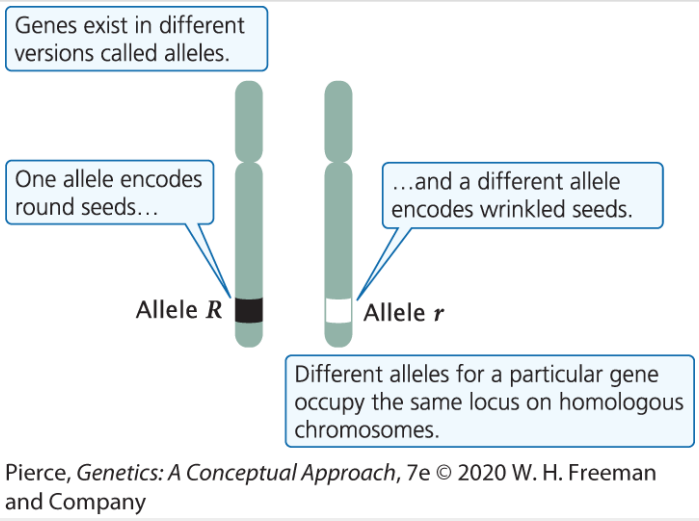
TABLE 3.1 Summary of important genetic terms

Term	Definition
Gene	An inherited factor (encoded in the DNA) that helps determine a characteristic
Allele	One of two or more alternative forms of a gene

Locus	A specific place on a chromosome occupied by an allele
Genotype	A set of alleles possessed by an individual organism
Homozygote	An individual organism possessing two of the same alleles at a locus
Heterozygote	An individual organism possessing two different alleles at a locus
Characteristic or character	An attribute or feature possessed by an organism
Phenotype or trait	The appearance or manifestation of a characteristic

Genes frequently come in different versions called **alleles** (**Figure 3.4**). In Mendel's crosses, seed shape was determined by a gene that exists as two different alleles: one allele encodes round seeds and the other encodes wrinkled seeds. All alleles for any particular gene will be found at a specific place on a chromosome, called the **locus** for that gene. (The plural of locus is *loci*; it's bad form in genetics—and incorrect—to speak of "locuses.") Thus, there is a specific place—a locus—on a chromosome in pea plants where the shape of seeds is determined. This locus may be occupied by an allele for round seeds

or an allele for wrinkled seeds. We will use the term *allele* when referring to a specific version of a gene; we will use the term *gene* to refer more generally to any allele at a locus.



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3.4 At each locus, a diploid organism possesses two alleles located on different homologous chromosomes. The alleles identified here refer to traits studied by Mendel.

Description
Allele big R is present at one locus on the chromosome on the left and encodes round seeds. Allele small R is present on the other chromosome on the right and encodes wrinkled seeds. Two caption captions read as follow: “Genes exist in different versions called alleles” and “Different alleles for a particular gene occupy the same locus on homologous chromosomes.”

The **genotype** is the set of alleles that an individual organism possesses. A diploid organism with a genotype consisting of two identical alleles is **homozygous** at that locus. One that has a genotype consisting of two different alleles is **heterozygous** at the locus.

A **phenotype** is the manifestation or appearance of a characteristic. This term can refer to any type of characteristic—physical, physiological, biochemical, or behavioral. Thus, the condition of having round seeds is a phenotype, a body weight of 50 kilograms (50 kg) is a phenotype, and having sickle-cell anemia is a phenotype. In this book, the term *characteristic* or *character* refers to a general feature, such as eye color; the term *trait* or *phenotype* refers to specific manifestations of that feature, such as blue or brown eyes.

A given phenotype arises from a genotype that develops within a particular environment. The genotype determines the potential for development; it sets certain limits, or boundaries, on that development. How the phenotype develops within those limits is determined by the effects of other genes and of environmental factors, and the balance between these effects varies from characteristic to characteristic. For some characteristics, differences between phenotypes are determined largely by differences in genotype. In Mendel's peas, for example, the genotype, not the environment, largely determined the shape of the seeds. For other characteristics, environmental differences are more important. The height reached by an oak tree at maturity is a phenotype that is strongly influenced by environmental factors, such as the availability of water, sunlight, and nutrients. Nevertheless, the tree's genotype imposes some limits on its height: an oak tree will never grow to be 300 meters (984 feet) tall, no matter how much sunlight, water, and fertilizer are provided. Thus, even the height of an oak tree is determined to some degree by genes. For many

characteristics, both genes and environment are important in determining phenotypic differences.

An obvious but important point is that only the alleles of the genotype are inherited. Although the phenotype is determined, at least to some extent, by the genotype, organisms do not transmit their phenotypes to the next generation. The distinction between genotype and phenotype is one of the most important principles of modern genetics. The next section describes Mendel's careful observation of phenotypes through several generations of breeding experiments. These experiments allowed him to deduce not only the genotypes of individual pea plants but also the rules governing their inheritance.

CONCEPTS

Each phenotype results from a genotype developing within a specific environment. The alleles of the genotype, not the phenotype, are inherited.

✔ CONCEPT CHECK 2

What is the difference between a locus and an allele? What is the difference between genotype and phenotype?

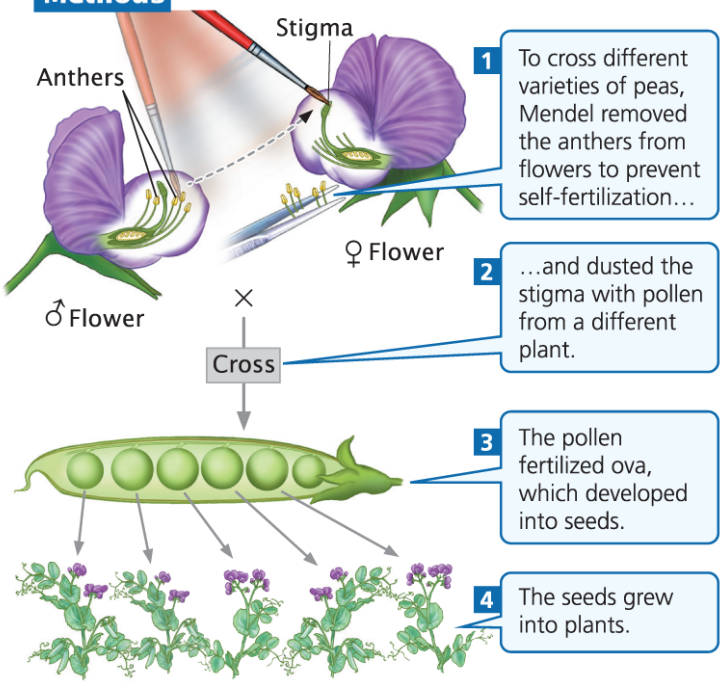
3.2 Monohybrid Crosses Reveal the Principle of Segregation and the Concept of Dominance

Mendel started with 34 varieties of peas and spent two years selecting those varieties that he would use in his experiments. He verified that each variety was pure-breeding (homozygous for each of the traits that he chose to study) by growing the plants for two generations and confirming that all offspring were the same as their parents. He then carried out a number of crosses between the different varieties. Although peas are normally self-fertilizing (each plant mates with itself), Mendel conducted crosses between different plants by opening the buds before the anthers (male sex organs) were fully developed, removing the anthers, and then dusting the stigma (female sex organ) with pollen from a different plant's anthers (**Figure 3.5**).

Experiment

Question: When peas with two different traits—round and wrinkled seeds—are crossed, will their progeny exhibit one of those traits, both of those traits, or an intermediate trait?

Methods



1. To cross different varieties of peas, Mendel removed the anthers from flowers to prevent self-fertilization…
2. …and dusted the stigma with pollen from a different plant.
3. The pollen fertilized ova, which developed into seeds.
4. The seeds grew into plants.

P generation Homozygous round seeds × Homozygous wrinkled seeds

5. Mendel crossed two homozygous varieties of peas.

Cross

F_1 generation

6. All the F_1 seeds were round. Mendel allowed plants grown from these seeds to self-fertilize.

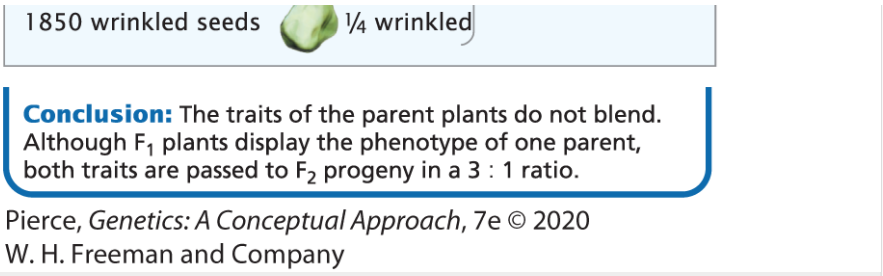
Self-fertilize

Results

F_2 generation

	Fraction of progeny seeds
5474 round seeds	¾ round

7. ¾ of F_2 seeds were round and ¼ were wrinkled, a 3 : 1 ratio.



3.5 Mendel conducted monohybrid crosses.

Description

The illustration shows the methods where a male flower with anthers and a female flower with a stigma are drawn. The first caption reads, “to cross different varieties of peas, Mendel removed the anthers from flowers to prevent self-fertilization.” Both these flowers are crossed and the second caption reads, “and dusted the stigma with pollen from a different plant.” A downward arrow shows a pea pod with peas and the third caption reads, “The pollen fertilized ova, which developed into seeds.” Each pea seed from the pod points to individual plants and the fourth caption reads, “The seeds grew into plants.” A cross is shown below this. P generation below shows homozygous round seeds crossed with homozygous wrinkled seeds. The fifth caption reads, “Mendel crossed two homozygous varieties of peas.” The F subscript 1 generation below produced from this cross shows four round peas which are self-fertilized. The sixth caption reads, “All the F subscript 1 seeds were round. Mendel allowed plants grown from these seeds to self-fertilize.” Results below show F subscript 2 generation in a fraction of progeny seeds. 5474 round seeds, three-fourth round and 1850 wrinkled seeds, one-fourth wrinkled. The seventh caption reads, “three-fourth of F subscript 2 seeds were round and one-fourth were wrinkled, a 3 to 1 ratio.” The conclusion states that the traits of the parent plants do not blend. Although F subscript 1 plants display the phenotype of one parent, both traits are passed to F subscript 2 progeny in a 3 to 1 ratio.

Mendel began by studying **monohybrid crosses**—crosses between parents that differed in a single characteristic. In one experiment, Mendel crossed a pea plant that was pure-breeding (homozygous) for round seeds with one that was pure-breeding for wrinkled seeds

(see Figure 3.5). This first generation of a cross is called the **P (parental) generation**.

After crossing the two varieties in the P generation, Mendel observed the offspring that resulted from the cross. The seed shape phenotype develops as soon as the seed matures because seed traits are determined by the newly formed embryo within the seed. For characteristics associated with the plant itself, such as stem length, the phenotype doesn't develop until the plant grows from the seed; for these characteristics, Mendel had to wait until the following spring, plant the seeds, and then observe the phenotypes of the plants that germinated.

The offspring of the parents in the P generation are the **F_1 (first filial) generation**. When Mendel examined the F_1 generation of this cross, he found that they expressed only one of the phenotypes present in the parental generation: all the F_1 seeds were round. Mendel carried out 60 such crosses and always obtained this result. Furthermore, he conducted **reciprocal crosses**: in one cross, pollen (the male gamete) was taken from a plant with round seeds, and in its reciprocal cross, pollen was taken from a plant with wrinkled seeds. Reciprocal crosses gave the same result: all the F_1 seeds were round.

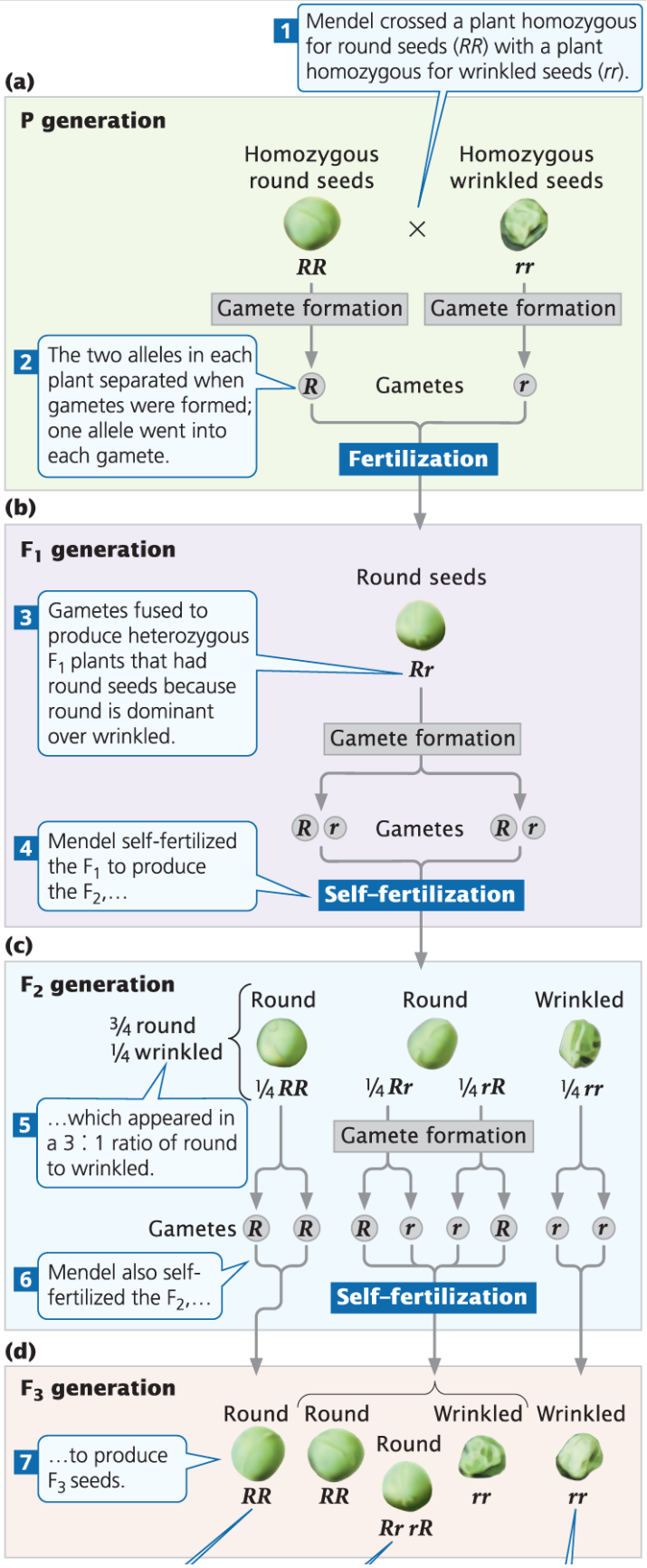
THINK-PAIR-SHARE Question 3

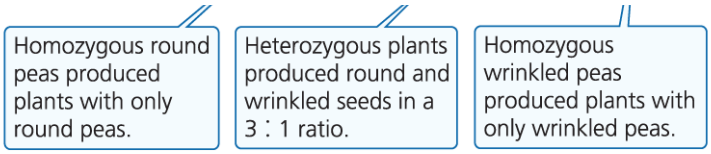
Mendel wasn't content with examining only the seeds arising from these monohybrid crosses, however. The following spring, he planted the F_1 seeds, cultivated the plants that germinated from them, and allowed the plants to self-fertilize, producing a second generation—the **F_2 (second filial) generation**. Both of the traits from the P generation emerged in the F_2 generation; Mendel counted 5474 round seeds and 1850 wrinkled seeds in the F_2 (see Figure 3.5). He noticed that the numbers of the round and wrinkled seeds constituted approximately a 3 to 1 ratio; that is, about $^3/_4$ of the F_2 seeds were round and $^1/_4$ were wrinkled. Mendel conducted monohybrid crosses for all seven of the characteristics that he studied in pea plants, and in all the crosses he obtained the same result: all of the F_1 resembled only one of the two parents, but both parental traits emerged in the F_2 in an approximate ratio of 3:1.

What Monohybrid Crosses Reveal

Mendel drew several conclusions from the results of his monohybrid crosses. First, he reasoned that although the F_1 plants display the phenotype of only one parent, they must inherit genetic factors from both parents because they transmit both parental phenotypes to the F_2 generation. The presence of both round and wrinkled seeds in the F_2 plants could be explained only if the F_1 plants possessed both round and wrinkled genetic factors that they had inherited from the P generation. He concluded that each plant must therefore possess two genetic factors encoding a characteristic.

The genetic factors (now called alleles) that Mendel discovered are, by convention, designated with letters: the allele for round seeds is usually represented by *R* and the allele for wrinkled seeds by *r*. The plants in the P generation of Mendel's cross possessed two identical alleles: *RR* in the round-seeded parent and *rr* in the wrinkled-seeded parent (**Figure 3.6a**).


1 Mendel crossed a plant homozygous for round seeds (RR) with a plant homozygous for wrinkled seeds (rr).
(a)
P generation
Homozygous round seeds
Homozygous wrinkled seeds
RR
rr
Gamete formation
Gamete formation
2 The two alleles in each plant separated when gametes were formed; one allele went into each gamete.
R
Gametes
r
Fertilization
(b)
F1 generation
Round seeds
3 Gametes fused to produce heterozygous F1 plants that had round seeds because round is dominant over wrinkled.
Rr
Gamete formation
R r
Gametes
R r
4 Mendel self-fertilized the F1 to produce the F2,…
Self-fertilization
(c)
F2 generation
Round
Round
Wrinkled
¾ round
¼ wrinkled
¼ RR
¼ Rr
¼ rR
¼ rr
5 …which appeared in a 3 : 1 ratio of round to wrinkled.
Gamete formation
Gametes
R R
R r r R
r r
6 Mendel also self-fertilized the F2,…
Self-fertilization
(d)
F3 generation
Round
Round
Wrinkled
Wrinkled
7 …to produce F3 seeds.
Round
RR
RR
rr
rr
Rr rR



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3.6 Mendel's monohybrid crosses revealed the principle of segregation and the concept of dominance.

Description

Part "a" at the top shows the P generation which consists of homozygous round seeds with genotype big R big R crossed with homozygous wrinkled seeds with genotype small R small R. The first caption reads, "Mendel crossed a plant homozygous for round seeds (big R big R) with a plant homozygous for wrinkled seeds (small R small R)." Gamete formation takes place with both the genotypes. Big R big R results in big R and small R small R results in small R. The second caption reads, "The two alleles in each plant separated when gametes were formed; one allele went into each gamete." Fertilization takes place and leads to part "b" below which represents F subscript 1 generation. F subscript 1 generation consists of big R small R progeny. The third caption reads, "Gametes fused to produce heterozygous F subscript 1 plants that had round seeds because round is dominant over wrinkled." Gamete formation takes place which includes big R small R and are self-fertilized. The fourth caption reads, "Mendel self-fertilized the F subscript 1 to produce the F subscript 2," It further leads to part "c" below which represents F subscript 2 generation. The F subscript 2 generation includes one-fourth big R big R round, one-fourth big R small R round, one-fourth small R big R round, and one-fourth small R small R wrinkled. These are three-fourth round and one-fourth wrinkled and the fifth caption reads, "which appeared in a 3 to 1 ratio of round to wrinkled." Gametes produced are big R and big R, big R and small R, small R and big R, and small R and small R. The sixth caption reads, "Mendel also self-fertilized the F subscript 2." Self-fertilization takes place which leads to part "d." Part "d" below shows the F subscript 3 generation. The seventh caption reads, "to produce F subscript 3 generation." The F subscript 3 generation include big R big R round (homozygous round peas produced plants with only round peas), big R big R, big R small R, small R big R and small R small R (Heterozygous plants produced round and wrinkled seeds in a 3 to 1 ratio), and small R small R (Homozygous wrinkled peas produced plants with only wrinkled peas.)

The second conclusion that Mendel drew from his monohybrid crosses was that the two alleles in each plant separate when gametes are formed, and one allele goes into each gamete. When two gametes (one from each parent) fuse to produce a zygote, the allele from the male parent unites with the allele from the female parent to produce the genotype of the offspring. Thus, Mendel's F_1 plants inherited an *R* allele from the round-seeded plant and an *r* allele from the wrinkled-seeded plant (**Figure 3.6b**). However, only the trait encoded by the round allele (*R*) was observed in the F_1: all the F_1 progeny had round seeds. Those traits that appeared unchanged in the F_1 heterozygous offspring Mendel called **dominant**, and those traits that disappeared in the F_1 heterozygous offspring he called **recessive**. In plants, alleles for dominant traits are often symbolized with uppercase letters (e.g., *R*), while alleles for recessive traits are often symbolized with lowercase letters (e.g., *r*). When dominant and recessive alleles are present together, the effect of the recessive allele is not observed in the phenotype. The concept of dominance was the third important conclusion that Mendel derived from his monohybrid crosses.

Mendel's fourth conclusion was that the two alleles of an individual plant separate with equal probability into the gametes. When plants of the F_1 (with genotype *Rr*) produced gametes, half of the gametes received the *R* allele for round seeds and half received the *r* allele for wrinkled seeds. The gametes then paired randomly to produce the following genotypes in equal proportions among the F_2: *RR*, *Rr*, *rR*,

rr (Figure 3.6c). Because round (*R*) is dominant to wrinkled (*r*), there were three round-seeded progeny (*RR*, *Rr*, *rR*) for every wrinkled-seeded progeny (*rr*) in the F_2. This 3:1 ratio of round-seeded to wrinkled-seeded progeny that Mendel observed in the F_2 could be obtained only if the two alleles of a genotype separated into the gametes with equal probability.

The conclusions that Mendel drew about inheritance from his monohybrid crosses have been further developed and formalized into the principle of segregation and the concept of dominance. The **principle of segregation** (**Mendel's first law**; see Table 3.2) states that each individual diploid organism possesses two alleles for any particular characteristic, one inherited from the maternal parent and one from the paternal parent. These two alleles segregate (separate) when gametes are formed, and one allele goes into each gamete. Furthermore, the two alleles segregate into gametes in equal proportions. The **concept of dominance** states that when two different alleles are present in a genotype, only the trait encoded by one of them—the dominant allele—is observed in the phenotype.

TABLE 3.2 Comparison of the principles of segregation and independent assortment

Principle	Observation	Stage of Meiosis*
Segregation (Mendel's first	1. Each individual organism possesses	Before meiosis

law)	two alleles encoding a trait.	
	2. Alleles separate when gametes are formed.	Anaphase I
	3. Alleles separate in equal proportions.	Anaphase I
Independent assortment (Mendel's second law)	Alleles at different loci separate independently.	Anaphase I

*Assumes that no crossing over occurs. If crossing over takes place, then segregation and independent assortment may also occur in anaphase II of meiosis.

Mendel confirmed these principles by allowing his F_2 plants to self-fertilize and produce an F_3 generation. He found that the plants grown from the wrinkled seeds—those displaying the recessive trait (*rr*)—produced an F_3 in which all plants produced wrinkled seeds. Because his wrinkled-seeded plants were homozygous for wrinkled alleles (*rr*), only wrinkled alleles could be passed on to their progeny (**Figure 3.6d**).

The plants grown from round seeds—the dominant trait—fell into two types (see Figure 3.6c). Following self-fertilization, about $^2/_3$ of these plants produced both round-seeded and wrinkled-seeded progeny in the F_3 generation. These plants were heterozygous (*Rr*),

so they produced $^1/_4$ *RR* (round), $^1/_2$ *Rr* (round), and $^1/_4$ *rr* (wrinkled) progeny, giving a 3:1 ratio of round to wrinkled in the F_3. About $^1/_3$ of the plants grown from round seeds were of the second type; they produced only the round-seeded trait in the F_3. These plants were homozygous for the round allele (*RR*) and could thus produce only round-seeded offspring in the F_3 generation. Mendel planted the seeds obtained in the F_3 and carried these plants through three more rounds of self-fertilization. In each generation, $^2/_3$ of the round-seeded plants produced round and wrinkled offspring, whereas $^1/_3$ produced only round offspring. These results are entirely consistent with the principle of segregation.

CONCEPTS

The principle of segregation states that each individual organism possesses two alleles that encode a characteristic. These alleles segregate when gametes are formed, and one allele goes into each gamete. The concept of dominance states that when the two alleles of a genotype are different, only the trait encoded by the dominant allele is observed.

✔ CONCEPT CHECK 3

How did Mendel know that each of his pea plants carried two alleles encoding a characteristic?

The Molecular Nature of Alleles

What exactly is an allele, and how does it determine a phenotype? Although Mendel had no information about the physical nature of the genetic factors in his crosses, modern geneticists have now determined the molecular basis of those factors and how they encode a trait such as wrinkled peas.

Alleles, such as the *R* and *r* alleles that encode round and wrinkled peas, usually represent specific DNA sequences. The locus that determines whether a pea is round or wrinkled is a sequence of DNA on pea chromosome 5 that encodes a protein called starch-branching enzyme isoform I (SBEI). The *R* allele, which produces round seeds in pea plants, encodes a normal, functional form of the SBEI enzyme. This enzyme converts a linear form of starch into a highly branched form. The *r* allele, which encodes wrinkled seeds, is a different DNA sequence that contains a mutation or error; it encodes an inactive form of the enzyme that does not produce the branched form of starch and leads to the accumulation of sucrose within the *rr* seed (the pea). Because the *rr* seed contains a large amount of sucrose, the developing seed absorbs water and swells. Later, as the seed matures, it loses water. Because *rr* seeds absorb more water and expand more during development, they lose more water during maturation and afterward appear shriveled or wrinkled. The *r* allele for wrinkled seeds is recessive because the presence of a single *R* allele in the heterozygote allows the plant to synthesize enough SBEI enzyme to produce branched starch and, therefore, round seeds.

Research has revealed that the *r* allele contains an extra 800 base pairs of DNA, which disrupt the normal coding sequence of the gene. The extra DNA appears to have come from a transposable element, a type of DNA sequence that has the ability to move from one location in the genome to another, which we will discuss further in Chapter 18.

CONNECTING CONCEPTS

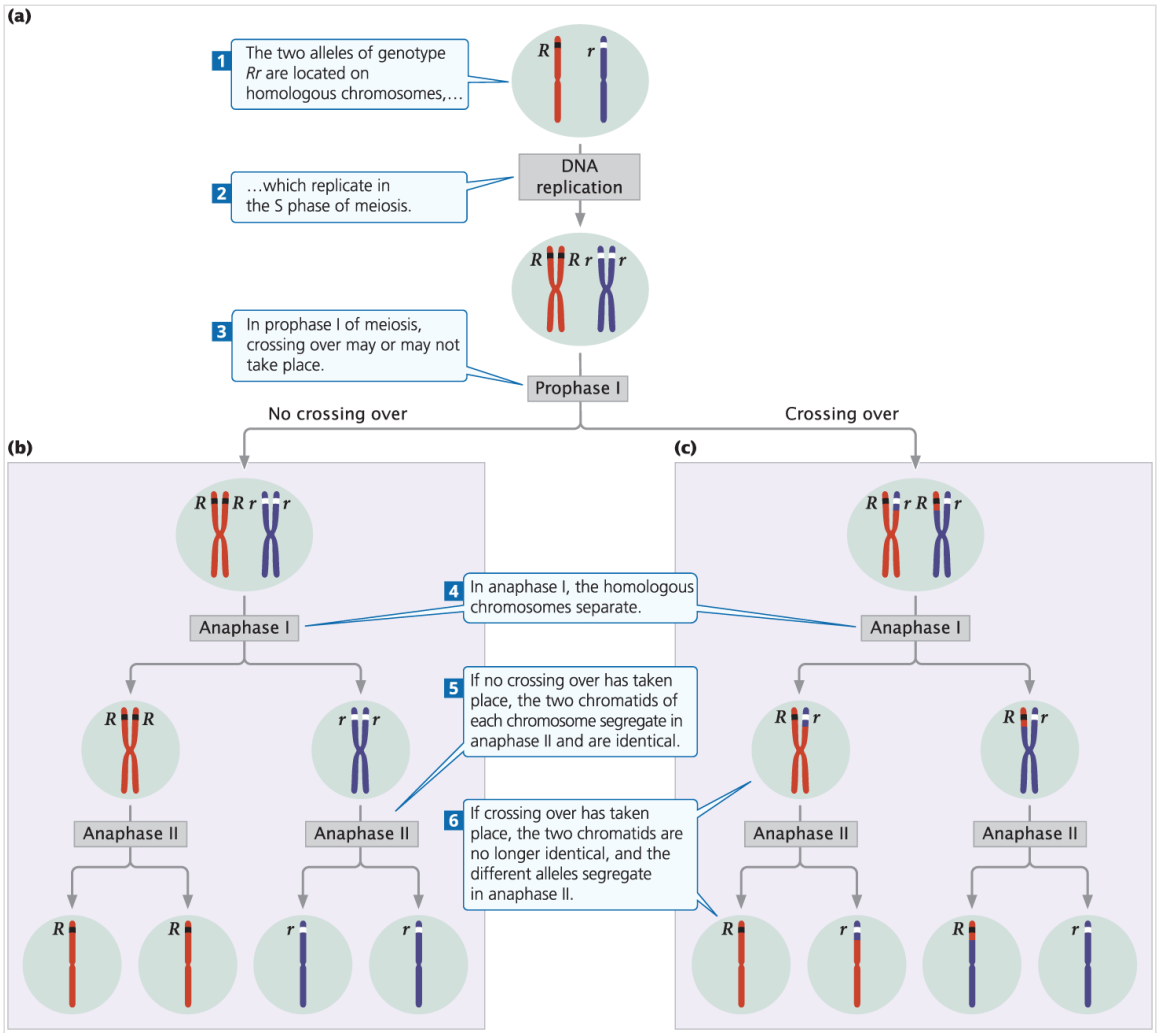
Relating Genetic Crosses to Meiosis

We have now seen how the results of monohybrid crosses are explained by Mendel's principle of segregation. Many students find that they enjoy working genetic crosses but are frustrated by the abstract nature of the symbols. Perhaps you feel the same at this point. You may be asking, "What do these symbols really represent? What does the genotype *RR* mean in regard to the biology of the organism?" The answers to these questions lie in relating the abstract symbols of crosses to the structure and behavior of chromosomes, the repositories of genetic information (see Chapter 2).

In 1900, when Mendel's work was rediscovered and biologists began to apply his principles of heredity, the relation between genes and chromosomes was still unclear. The theory that genes are located on chromosomes (the **chromosome theory of heredity**) was developed in the early 1900s by Walter Sutton, then a graduate student at Columbia University. Through the careful study of meiosis in insects, Sutton documented the fact that each homologous pair of chromosomes consists of one maternal chromosome and one paternal chromosome. Showing that these pairs segregate independently into gametes in meiosis, he concluded that this process is the biological basis for Mendel's principles of heredity. German cytologist and embryologist Theodor Boveri came to similar conclusions at about the same time.

The symbols used in genetic crosses, such as *R* and *r*, are just shorthand notations for particular sequences of DNA in the chromosomes that encode particular phenotypes. The two alleles of a genotype are found on different but homologous chromosomes. One chromosome of each homologous pair is inherited from the mother, and the other is inherited from the father. In the S phase of meiotic interphase, each chromosome replicates, producing two

copies of each allele, one on each chromatid (**Figure 3.7a**). The homologous chromosomes segregate in anaphase I, thereby separating the two different alleles (**Figure 3.7b and c**). This chromosome segregation is the basis of the principle of segregation. In anaphase II of meiosis, the two chromatids of each replicated chromosome separate, so each gamete resulting from meiosis carries only a single allele at each locus, as Mendel's principle of segregation predicts. If crossing over has taken place in prophase I of meiosis, then the two chromatids of each replicated chromosome are no longer identical, and the segregation of different alleles takes place at anaphase I and anaphase II (see Figure 3.7c).



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3.7 Segregation results from the separation of homologous chromosomes in meiosis.

Description

Part “a” on the top contains a cell with a rod-shaped maternal chromatid in red with big R gene and a rod-shaped paternal chromatid in blue with small R gene. A caption reads, “the two alleles of genotype big R small R are located on homologous chromosomes, which replicate in the S phase of meiosis.” D N A replication occurs and the next cell shows a red and a blue X-shaped chromosome. The caption reads: in prophase 1 of meiosis, crossing over may or may not take place. Below the cell with the X-shaped chromosomes, are 2 arrows showing the results of meiosis without and with cross over in part “b” and “c”, respectively. When there is no cross over, one X-shaped chromosome is 100 percent paternal and the other X-shaped chromosome is 100 percent maternal. At anaphase 1, the sister chromatids are pulled apart, leading to 2 cells. One cell has maternal chromatids with big R alleles and 1 cell has paternal chromatids with small R alleles. Part “c” on the right shows the results where crossing over did occur. There is a maternal X-shaped chromosome and a paternal X-shaped chromosome that are both heterozygous for the R allele. The caption reads as follows: in anaphase 1, the homologous chromosomes separate. If no crossing over has taken place, the 2 chromatids of each chromosome segregate in anaphase 2 and are identical. If crossing over has taken place, the 2 chromatids are no longer identical, and the different alleles segregate in anaphase 2. At the end of anaphase 2, the diagram on the left shows the 4 chromatids segregate when no crossing over takes and are identical as parents. The diagram on the right shows the segregation of 4 chromatids but 2 of them are not identical to the parents. Two of the chromatids resemble the parents, with a maternal strand with a big R allele and a paternal chromosome with a small R allele. The other two are hybrids and have the opposite allele of the parent strand.

Mendel knew nothing about chromosomes; he formulated his principles of heredity entirely on the basis of the results of the crosses that he carried out. Nevertheless, we should not forget that these principles work because they are based on the behavior of actual chromosomes in meiosis. ❯ **TRY PROBLEM 30**

Predicting the Outcomes of Genetic Crosses

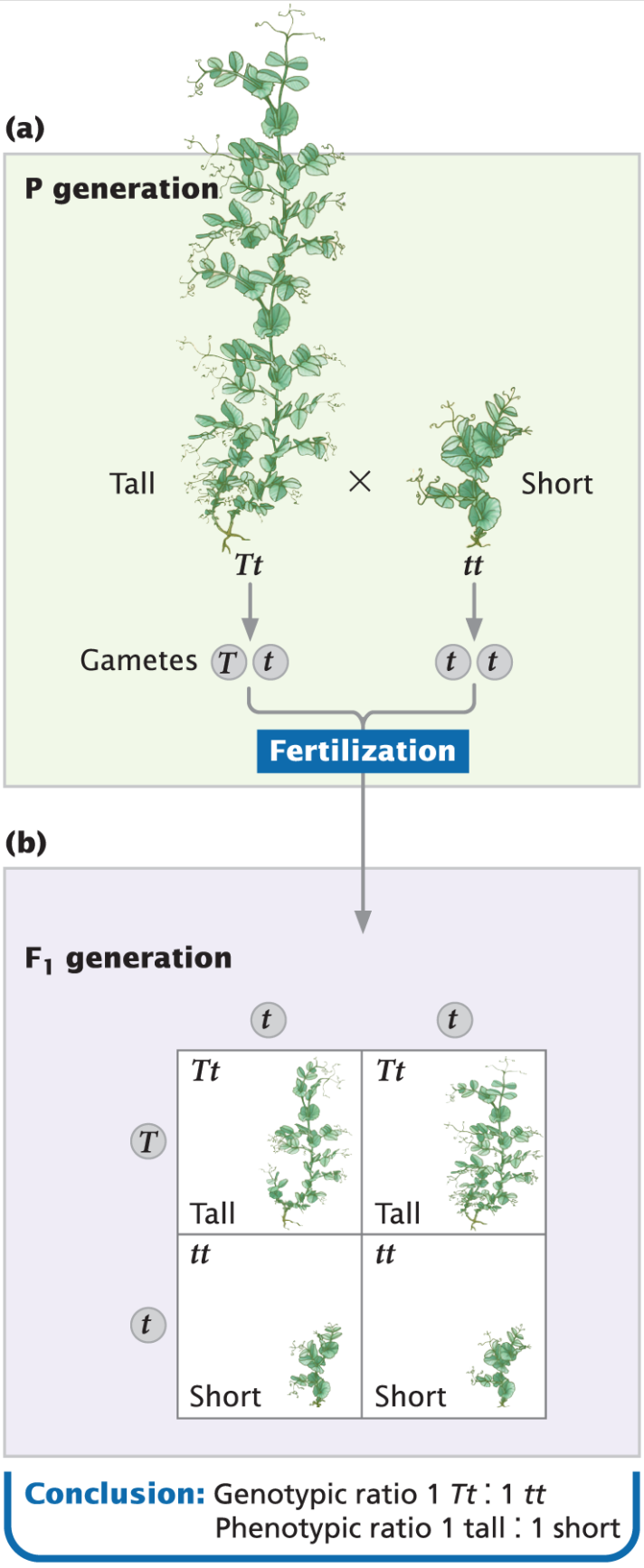
One of Mendel's goals in conducting his experiments in pea plants was to develop a way to predict the outcomes of crosses between plants with different phenotypes. In this section, you will first learn a simple shorthand method for predicting outcomes of genetic crosses (the Punnett square), and then you will learn how to use probability to predict the results of crosses.

THE PUNNETT SQUARE

The Punnett square was developed by the English geneticist Reginald C. Punnett in 1917. To illustrate the Punnett square, let's examine another cross carried out by Mendel. By crossing two varieties of pea plants that differed in height, Mendel established that tall (*T*) was dominant to short (*t*). He tested his theory concerning the inheritance of dominant traits by crossing an F_1 tall plant that was heterozygous (*Tt*) with the short homozygous parental variety (*tt*). This type of cross, between an F_1 genotype and either of the parental genotypes, is called a **backcross**.

To predict the types of offspring that will result from this backcross, we must first determine which gametes will be produced by each parent (Figure 3.8a). The principle of segregation tells us that the two alleles in each parent separate and that one allele passes to each

gamete. All gametes from the homozygous *tt* short plant will receive a single short (*t*) allele. The tall plant in this cross is heterozygous (*Tt*), so 50% of its gametes will receive a tall allele (*T*) and the other 50% will receive a short allele (*t*).


(a)
P generation
Tall
×
Short
Tt
tt
Gametes
T
t
t
t
Fertilization
(b)
F1 generation
t
t
T
t
Tt
Tall
Tt
Tall
tt
Short
tt
Short
Conclusion: Genotypic ratio 1 Tt : 1 tt
Phenotypic ratio 1 tall : 1 short

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3.8 The Punnett square can be used to determine the results of a genetic cross.

Description

Part “a” of the illustration on the top shows P generation. Drawings of a tall and a short pea plant are shown. A cross between a tall plant with genotype big T small T and a short plant with genotype small T small T takes place. The gametes formed are as follows: big T, small T, small T, and small T. Fertilization occurs and leads to part “b” below which demonstrates the F subscript 1 generation with a Punnett square, a table with 2 rows and 2 columns, with each row and column labeled with gametes. The data reads as follows:

Row 1 column 1: big T small T, tall

Row 1 column 2: big T small T, tall

Row 2 column 1: small T small T, short

Row 2 column 2: small T small T, short

A **Punnett square** is constructed by drawing a grid, listing the gametes produced by one parent along the upper edge, and listing the gametes produced by the other parent down the left side (**Figure 3.8b**). Each cell (that is, each block within the Punnett square) is then filled in with an allele from each of the corresponding gametes, generating the genotype of the progeny produced by the fusion of those gametes. In the upper left-hand cell of the Punnett square in Figure 3.8b, a gamete containing *T* from the tall plant unites with a gamete containing *t* from the short plant, giving the genotype of the progeny (*Tt*). It is useful to write the phenotype expressed by each genotype; here, the progeny will be tall because the tall allele is

dominant to the short allele. This process is repeated for all the cells in the Punnett square.

By simply counting, we can determine the types of progeny produced and their ratios. In Figure 3.8b, two cells contain tall (*Tt*) progeny and two cells contain short (*tt*) progeny, so the genotypic ratio expected for this cross is 2 *Tt* to 2 *tt* (a 1:1 ratio). Another way to express this result is to say that we expect $^1/_2$ of the progeny to have genotype *Tt* (and the tall phenotype) and $^1/_2$ of the progeny to have genotype *tt* (and the short phenotype).

In this cross, the genotypic ratio and the phenotypic ratio are the same, but this outcome need not be the case for all crosses. Try completing a Punnett square for the cross in which the F_1 round-seeded plants in Figure 3.6b undergo self-fertilization (you should obtain a phenotypic ratio of 3 round to 1 wrinkled and a genotypic ratio of 1 *RR* to 2 *Rr* to 1 *rr*).

CONCEPTS

The Punnett square is a shorthand method of predicting the genotypic and phenotypic ratios of progeny from a genetic cross.

✔ CONCEPT CHECK 4

If the F_1 plant depicted in Figure 3.6b is backcrossed to the parent with round seeds, what proportion of the progeny will have wrinkled seeds? (Use a Punnett square.)

a. $^3/_4$

b. $1/2$
c. $1/4$
d. 0

PROBABILITY AS A TOOL OF GENETICS

Another method for determining the outcome of a genetic cross is to use the rules of probability, as Mendel did with his crosses. **Probability** expresses the likelihood of the occurrence of a particular event. It is the number of times that a particular event takes place, divided by the number of all possible outcomes. For example, a deck of 52 cards contains only one king of hearts. The probability of drawing one card from the deck at random and obtaining the king of hearts is $1/52$ because there is only one card that is the king of hearts (one event) and there are 52 cards that can be drawn from the deck (52 possible outcomes). The probability of drawing a card and obtaining an ace is $4/52$ because there are four cards that are aces (four events) and 52 cards (possible outcomes). Probability can be expressed either as a fraction ($4/52$ in this case) or as a decimal number (0.077 in this case).

The probability of a particular event may be determined by knowing something about *how* or *how often* the event takes place. We know, for example, that the probability of rolling a six-sided die and getting a four is $1/6$ because the die has six sides and any one side is equally likely to end up on top. So, in this case, understanding the nature of

the event—the shape of the thrown die—allows us to determine its probability. In other cases, we determine the probability of an event by making a large number of observations. When a weather forecaster says that there is a 40% chance of rain on a particular day, this probability was obtained by observing a large number of days with similar atmospheric conditions and finding that it rains on 40% of those days. In this case, the probability has been determined empirically (by observation).

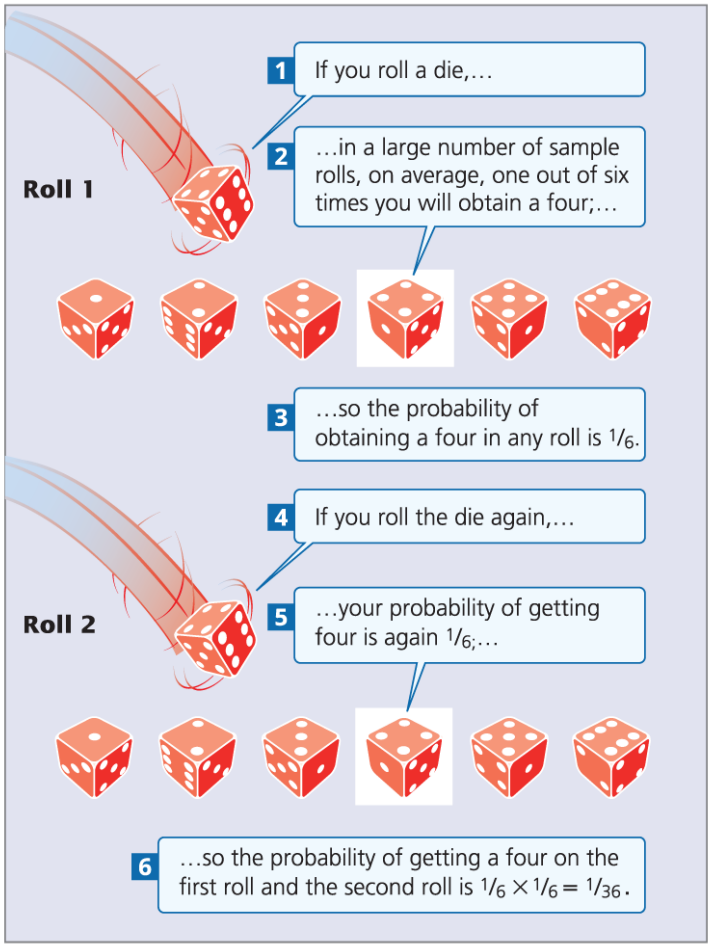
THE MULTIPLICATION RULE

Two rules of probability are useful for predicting the ratios of offspring from genetic crosses. The first is the **multiplication rule**, which states that the probability of two or more independent events taking place together is calculated by multiplying their independent probabilities.

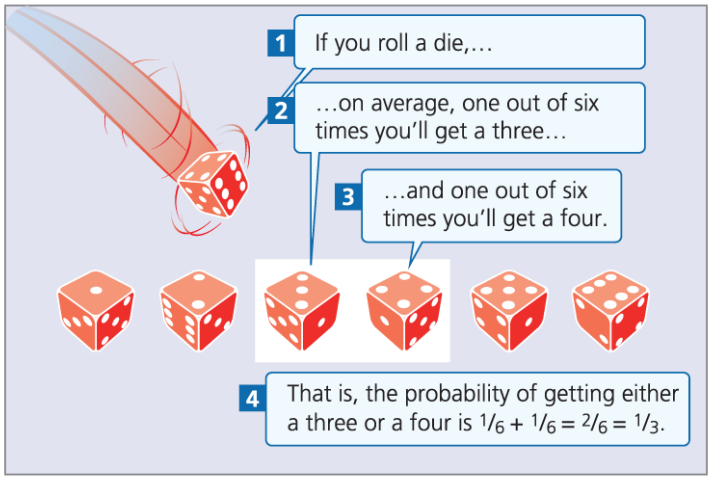
To illustrate the use of the multiplication rule, let's again consider the roll of a die. The probability of rolling one die and obtaining a four is $^1/_6$. To calculate the probability of rolling a die twice and obtaining two fours, we can apply the multiplication rule. The probability of obtaining a four on the first roll is $^1/_6$ and the probability of obtaining a four on the second roll is $^1/_6$, so the probability of rolling a four on both is $^1/_6 \times {}^1/_6 = {}^1/_{36}$ (**Figure 3.9a**). The key indicator for applying the multiplication rule is the word *and*; in the example just considered, we wanted to know the

probability of obtaining a four on the first roll *and* a four on the second roll. (It may have been some time since you worked with fractions. If you are rusty and need a review, turn to the appendix at the end of the book entitled Working with Fractions.)

(a) The multiplication rule



(b) The addition rule



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3.9 The multiplication and addition rules can be used to determine the probability of combinations of events.

Description

Part “a” on top shows the multiplication rule. It shows pictures of a die rolled twice. With both rolls, there is also a drawing of each side of the 6-sided die. In both pictures, the die with 4 on top is highlighted. The caption in the panel read as follows, “If you roll a die, in a large number of sample rolls, on average, one out of six times you will obtain a four, so the probability of obtaining a four in any roll is one-sixth. If you roll the die again, your probability of getting four is again one-sixth, so the probability of getting a 4 on the first roll and the second roll is one-sixth multiplied by one-sixth equals one over thirty six.

Part “b” below shows the addition rule. It also shows pictures of a die being rolled, and six pictures showing all 6 sides of a die individually. The die with 3 and 4 on top are highlighted. The caption read as follows, “If you roll a die, on average, 1 out of 6 times you’ll get a 3 and one out of six times you’ll get a four. That is, the probability of getting either a 3 or a 4 is one-sixth plus one-sixth equals two-sixths or one-third.

For the multiplication rule to be valid, the events whose joint probability is being calculated must be independent—the outcome of one event must not influence the outcome of the other. For example, the number that comes up on one roll of the die has no influence on the number that comes up on the next roll, so these events are independent. However, if we wanted to know the probability of being hit on the head with a hammer and going to the hospital on the same day, we could not simply apply the multiplication rule and multiply the two probabilities together because the two events are not independent—being hit on the head with a hammer certainly influences the probability of going to the hospital.

THE ADDITION RULE

The second rule of probability frequently used in genetics is the **addition rule**, which states that the probability of any of two or more mutually exclusive events is calculated by adding the probabilities of the events. Let's look at this rule in concrete terms. To obtain the probability of throwing a die once and rolling *either* a three *or* a four, we would use the addition rule, adding the probability of obtaining a three ($^1/_6$) to the probability of obtaining a four (again, $^1/_6$), or $^1/_6 + {}^1/_6 = {}^2/_6 = {}^1/_3$ (**Figure 3.9b**). The key indicators for applying the addition rule are the words *either* and *or*.

For the addition rule to be valid, the events whose probability is being calculated must be mutually exclusive, meaning that one event excludes the possibility of the other. For example, you cannot throw a single die just once and obtain both a three and a four because only one side of the die can be on top. These events are mutually exclusive.

CONCEPTS

The multiplication rule states that the probability of two or more independent events taking place together is calculated by multiplying their independent probabilities. The addition rule states that the probability that any of two or more mutually exclusive events taking place is calculated by adding their probabilities.

✔ CONCEPT CHECK 5

If the probability of being blood type A is $^1/_8$ and the probability of being blood type O is $^1/_2$, what is the probability of being either blood type A or blood type O?

a. $^{5}/_{8}$
b. $^{1}/_{2}$
c. $^{1}/_{10}$
d. $^{1}/_{16}$

APPLYING PROBABILITY TO GENETIC CROSSES

The multiplication and addition rules of probability can be used in place of the Punnett square to predict the ratios of progeny expected from a genetic cross. Let's first consider a cross between two pea plants heterozygous for the locus that determines height, *Tt* × *Tt*. Half of the gametes produced by each plant have a *T* allele, and the other half have a *t* allele, so the probability for each type of gamete is $^{1}/_{2}$.

The gametes from the two parents can combine in four different ways to produce offspring. Using the multiplication rule, we can determine the probability of each possible combination. To calculate the probability of obtaining *TT* progeny, for example, we multiply the probability of receiving a *T* allele from the first parent ($^{1}/_{2}$) by the probability of receiving a *T* allele from the second parent ($^{1}/_{2}$). The multiplication rule should be used here because we need the probability of receiving a *T* allele from the first parent *and* a *T* allele from the second parent—two independent events. The four types of progeny from this cross and their associated probabilities are

TT	(*T* gamete and *T* gamete)	$^1/_2 \times {}^1/_2 = {}^1/_4$	tall
Tt	(*T* gamete and *t* gamete)	$^1/_2 \times {}^1/_2 = {}^1/_4$	tall
tT	(*t* gamete and *T* gamete)	$^1/_2 \times {}^1/_2 = {}^1/_4$	tall
tt	(*t* gamete and *t* gamete)	$^1/_2 \times {}^1/_2 = {}^1/_4$	short

Notice that there are two ways for heterozygous progeny to be produced: a heterozygote can either receive a *T* allele from the first parent and a *t* allele from the second or receive a *t* allele from the first parent and a *T* allele from the second.

After determining the probabilities of obtaining each progeny genotype, we can use the addition rule to determine the overall phenotypic ratios. Because of dominance, a tall plant can have genotype *TT*, *Tt*, or *tT*, so, using the addition rule, we find the probability of tall progeny to be $^1/_4 + {}^1/_4 + {}^1/_4 = {}^3/_4$. Because only one genotype (*tt*) encodes the short phenotype, the probability of short progeny is simply $^1/_4$.

Two methods have now been introduced to work genetic crosses: the Punnett square and the probability method. At this point, you may

be asking, "Why bother with probability rules and calculations? The Punnett square is easier to understand and just as quick." This is true for simple monohybrid crosses. For tackling more complex crosses that assess genes at two or more loci, however, the probability method is both clearer and quicker than the Punnett square.

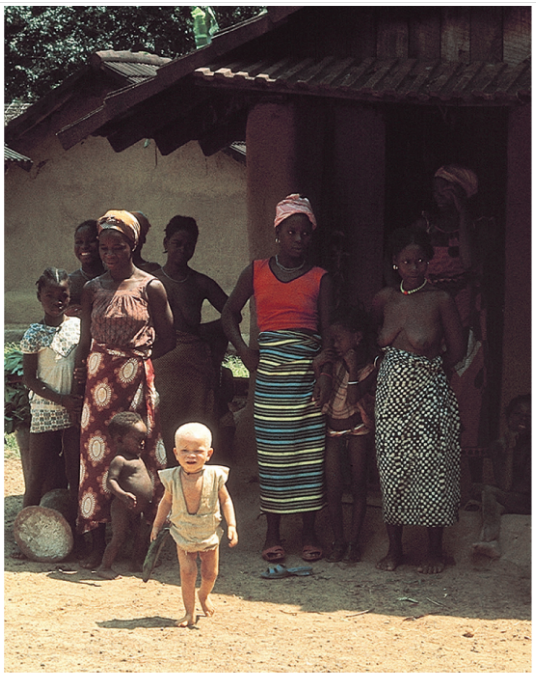
CONDITIONAL PROBABILITY

Thus far, we have used probability to predict the chances of producing certain types of progeny given only the genotypes of the parents. Sometimes, however, we have additional information that modifies, or *conditions*, the probability, a situation termed **conditional probability**. For example, assume that we cross two heterozygous pea plants (*Tt* × *Tt*) and obtain a tall progeny plant. What is the probability that this tall plant is heterozygous (*Tt*)? You might assume that the probability would be $^1/_2$, the probability of obtaining heterozygous progeny in a cross between two heterozygotes. In this case, however, we have some additional information—the phenotype of the progeny plant—which modifies that probability. When two heterozygous individuals are crossed, we expect $^1/_4$ *TT*, $^1/_2$ *Tt*, and $^1/_4$ *tt* progeny. We know that the plant in question is tall, so we can eliminate the possibility that it has genotype *tt*. Tall progeny must be either genotype *TT* or genotype *Tt*, and in a cross between two heterozygotes, these genotypes occur in

a 1:2 ratio. Therefore, the probability that a tall progeny plant is heterozygous (*Tt*) is two out of three, or $^{2}/_{3}$.

THE BINOMIAL EXPANSION AND PROBABILITY

When probability is used, it is important to recognize that there may be several different ways in which a set of events can occur. Consider two parents who are both heterozygous for albinism, a recessive condition in humans that causes reduced pigmentation in the skin, hair, and eyes (**Figure 3.10**; see also the introduction to Chapter 1). When two parents heterozygous for albinism mate ($Aa \times Aa$), the probability of their having a child with albinism (aa) is $^{1}/_{4}$, and the probability of their having a child with full pigmentation (AA or Aa) is $^{3}/_{4}$. Suppose we want to know the probability of this couple having three children with albinism. In this case, there is only one way in which this can happen: their first child has albinism *and* their second child has albinism *and* their third child has albinism. Here, we simply apply the multiplication rule: $^{1}/_{4} \times {}^{1}/_{4} \times {}^{1}/_{4} = {}^{1}/_{64}$.



Richard Hutchings/Science Source.

3.10 Albinism in humans is usually inherited as a recessive trait.

Suppose we now ask what the probability is of this couple having three children, one with albinism and two with pigmentation. This situation is more complicated. The first child might have albinism, whereas the second and third might be unaffected; the probability of this sequence of events is $^1/_4 \times {}^3/_4 \times {}^3/_4 = {}^9/_{64}$. Alternatively, the first and third children might have pigmentation, whereas the second might have albinism; the probability of this sequence is $^3/_4 \times {}^1/_4 \times {}^3/_4 = {}^9/_{64}$. Finally, the first two children might have pigmentation and the third albinism; the probability of this sequence is $^3/_4 \times {}^3/_4 \times {}^1/_4 = {}^9/_{64}$. Because *either* the first sequence *or* the second sequence *or* the third sequence produces one child with albinism and two with pigmentation, we apply the addition rule and add the probabilities: $^9/_{64} + {}^9/_{64} + {}^9/_{64} = {}^{27}/_{64}$.

If we want to know the probability of this couple having five children, two with albinism and three with pigmentation, figuring out *all* the different combinations of children and their probabilities becomes more difficult. This task is made easier if we apply the binomial expansion.

The binomial takes the form $(p + q)^n$, where p equals the probability of one event, q equals the probability of the alternative event, and n equals the number of times the event occurs. For figuring the probability of two out of five children with albinism,

$$p = \text{the probability of a child having albinism } (^1/_4)$$
$$q = \text{the probability of a child having pigmentation } (^3/_4)$$

The binomial for this situation is $(p + q)^5$ because there are five children in the family ($n = 5$). The expansion of this binomial is

$$(p+q)^5 = p^5 + 5p^4q + 10p^3q^2 + 10p^2q^3 + 5pq^4 + q^5$$

Each of the terms in the expansion provides the probability of one particular combination of traits in the children. The first term in the expansion (p^5) equals the probability of having five children with albinism because p is the probability of albinism. The second term ($5p^4q$) equals the probability of having four children with albinism and one with pigmentation, the third term ($10p^3q^2$) equals the probability of having three children with albinism and two with pigmentation, and so forth.

To obtain the probability of any combination of events, we insert the values of p and q. Thus, the probability of having two out of five children with albinism is

$$10p^2q^3 = 10\ (^1/_4)^2\ (^3/_4)^3 = {}^{270}/_{1024} = 0.26$$

We can easily figure out the probability of any desired combination of albinism and pigmentation among five children by using the other terms in the expansion.

How did we expand the binomial in this example? In general, the expansion of any binomial $(p + q)^n$ consists of a series of $n + 1$ terms. In the preceding example, $n = 5$, so there are 5 + 1 = 6 terms: p^5, $5p^4q$, $10p^3q^2$, $10p^2q^3$, $5pq^4$, and q^5. To write out the terms, first figure out their exponents. The exponent of p in the first term is always the power to which the binomial is raised, or n. In our example, $n = 5$, so our first term is p^5. The exponent of p decreases by one in each successive term, so the exponent of p is 4 in the second term (p^4), 3 in the third term (p^3), and so forth. The exponent of q is 0 (no q) in the first term and increases by one in each successive term, increasing from 0 to 5 in our example.

Next, we determine the coefficient of each term. The coefficient of the first term is always 1, so in our example, the first term is $1p^5$, or just p^5. The coefficient of the second term is always the same as the power to which the binomial is raised; in our example, this coefficient is 5, and the term is $5p^4q$. For the coefficient of the third

term, we look back at the preceding term, multiply the coefficient of the preceding term (5 in our example) by the exponent of p in that term (4), and then divide by the number of that term (second term, or 2). So the coefficient of the third term in our example is $(5 \times 4)/2 = {}^{20}/_{2} = 10$, and the term is $10p^3q^2$. We follow this procedure for each successive term. The coefficients for the terms in the binomial expansion can also be determined from Pascal's triangle (Table 3.3). The exponents and coefficients for each term in the first five binomial expansions are given in Table 3.4.

TABLE 3.3 Pascal's triangle

The numbers in each row represent the coefficients of each term in the binomial expansion $(p + q)^n$.

n	Coefficients
	1
1	1 1
2	1 2 1
3	1 3 3 1
4	1 4 6 4 1
5	1 5 10 10 5 1
6	1 6 15 20 15 6 1

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Description

The table has the following column headers: n for first column and a collective header "coefficients" for second to thirteenth column. The row entries are as follows:

Row 1: No data; No data; No data; No data; No data; No data; No data; 1; No data; No data; No data; No data; No data; No data.

Row 2: 1; No data; No data; No data; No data; No data; 1; No data; 1; No data; No data; No data; No data; No data.

Row 3: 2; No data; No data; No data; No data; 1; No data; 2; No data; 1; No data; No data; No data; No data.

Row 4: 3; No data; No data; No data; 1 ; No data; 3 ; No data; 3; No data; 1; No data; No data; No data.

Row 5: 4; No data; No data; 1; No data; 4; No data; 6; No data; 4; No data; 1; No data; No data.

Row 6: 5; No data; 1; No data; 5; No data; 10; No data; 10; No data; 5; No data; 1; No data.

Row 7: 6; 1; No data; 6; No data; 15; No data; 20; No data; 15; No data; 6; No data; 1; No data.

Note: Each number in the triangle, except for the first, is equal to the sum of the two numbers directly above it.

TABLE 3.4 Coefficients and terms for the binomial expansion $(p + q)^n$ for $n = 1$ through 5

n	Binomial Expansion
1	$a + b$
2	$a^2 + 2ab + b^2$

3	$a^3 + 3a^2b + 3ab^2 + b^3$
4	$a^4 + 4a^3b + 6a^2b^2 + 4ab^3 + b^4$
5	$a^5 + 5a^4b + 10a^3b^2 + 10a^2b^3 + 5ab^4 + b^5$

Another way to determine the probability of any particular combination of events is to use the following formula:

$$P = \frac{n!}{s!t!}p^s q^t$$

where *P* equals the overall probability of event X with probability *p* occurring *s* times and alternative event Y with probability *q* occurring *t* times. For our albinism example, event X would be the occurrence of a child with albinism ($^1/_4$) and event Y would be the occurrence of a child with pigmentation ($^3/_4$); *s* would equal the number of children with albinism (2) and *t* would equal the number of children with pigmentation (3). The ! symbol stands for factorial, and it means the product of all the integers from *n* to 1. In this example, *n* = 5; so *n*! = 5 × 4 × 3 × 2 × 1. Applying this formula to obtain the probability of two out of five children having albinism, we obtain

$$P = \frac{5!}{2!3!}(^1/_4)^2(^3/_4)^3 = \frac{5 \times 4 \times 3 \times 2 \times 1}{2 \times 1 \times 3 \times 2 \times 1}(^1/_4)^2(^3/_4)^3 = 0.26$$

This value is the same as that obtained with the binomial expansion.

› TRY PROBLEMS 25, 26, AND 27

THINK-PAIR-SHARE Question 4

The Testcross

A useful tool for analyzing genetic crosses is the **testcross**, in which one individual of unknown genotype is crossed with another individual with a homozygous recessive genotype for the trait in question. Figure 3.8 illustrates a testcross (in this case, it is also a backcross). A testcross tests, or reveals, the genotype of the first individual.

Suppose you were given a tall pea plant with no information about its parents. Because tallness is a dominant trait in peas, your plant could be either homozygous (*TT*) or heterozygous (*Tt*) for the dominant allele, but you would not know which. You could determine its genotype by performing a testcross. If the plant were homozygous (*TT*), a testcross would produce all tall progeny (*TT* × *tt* → all *Tt*); if the plant were heterozygous (*Tt*), approximately half of the progeny would be tall and approximately half would be short (*TT* × *tt* → $^1/_2$ *Tt* and $^1/_2$ *tt*). When a testcross is performed, any recessive allele in the unknown genotype will be expressed in the progeny

because it will be paired with a recessive allele from the homozygous recessive parent. **› TRY PROBLEMS 18 AND 21**

THINK-PAIR-SHARE Question 5

CONCEPTS

The binomial expansion can be used to determine the probability of a particular set of events. A testcross is a cross between an individual with an unknown genotype and one with a homozygous recessive genotype. The outcome of the testcross can reveal the unknown genotype.

Genetic Symbols

As we have seen, genetic crosses are usually depicted with symbols that designate the different alleles. The symbols used for alleles are usually determined by the community of geneticists who work on a particular organism; therefore, there is no universal system for designating symbols. In plants, lowercase letters are often used to designate recessive alleles and uppercase letters to designate dominant alleles. Two or three letters may be used for a single allele: the recessive allele for heart-shaped leaves in cucumbers is designated *hl*, and the recessive allele for the abnormal sperm-head shape in mice is designated *azh*.

In animals, the most common allele for a characteristic—called the **wild type** because it is the allele usually found in the wild—is often symbolized by one or more letters and a plus sign (+). The letter or letters chosen are usually based on a mutant (less common) phenotype. For example, the recessive allele that encodes yellow eyes in the Oriental fruit fly is represented by *ye*, whereas the allele for wild-type eye color is represented by ye^+. At times, the letters for the wild-type allele are dropped and the allele is represented simply by a plus sign.

Gene names and abbreviations are usually italicized. Superscripts and subscripts are sometimes added to distinguish between genes: Lfr_1 and Lfr_2 represent dominant mutant alleles at different loci that produce lacerate leaf margins in opium poppies; El^R represents an allele in goats that restricts the length of the ears.

A slash may be used to distinguish the two alleles present in an individual genotype. For example, the genotype of a goat that is heterozygous for restricted ears might be written El^+/El^R, or simply $+/El^R$. If genotypes at more than one locus are presented together, a space separates the genotypes. For example, a goat heterozygous for a pair of alleles that produces restricted ears and heterozygous for another pair of alleles that produces goiter can be designated $El^+/El^RG/g$. Sometimes it is useful to designate the possibility of several genotypes. An underline in a genotype, such as $A_$, indicates

that any allele is possible. In this case, $A_$ might include both AA and Aa genotypes.

CONNECTING CONCEPTS

Ratios in Simple Crosses

Now that we have had some experience with genetic crosses, let's review the ratios that appear in the progeny of simple crosses, in which a single locus is under consideration and one of the alleles is dominant to the other. Understanding these ratios and the parental genotypes that produce them will enable you to work simple genetic crosses quickly, without resorting to the Punnett square. Later in this chapter, we use these ratios to work more complicated crosses that include several loci.

There are only three phenotypic ratios to understand (**Table 3.5**). The first is the 3:1 ratio, which arises in a simple genetic cross when both of the parents are heterozygous for a dominant trait (*Aa* × *Aa*). The second phenotypic ratio is the 1:1 ratio, which results from the mating of a heterozygous parent and a homozygous recessive parent. The homozygous parent in this cross must carry two recessive alleles (*Aa* × *aa*) because a cross between a homozygous dominant parent and a heterozygous parent (*AA* × *Aa*) produces offspring displaying only the dominant trait.

TABLE 3.5 Phenotypic ratios for simple genetic crosses (crosses for a single locus) with dominance

An underline in a genotype, such as *A*_, indicates that any allele is possible.

Phenotypic Ratio	Genotypes of Parents	Genotypes of Progeny
3:1	*Aa* × *Aa*	$^3/_4$ *A*_: $^1/_4$ *aa*
1:1	*Aa* × *aa*	$^1/_2$ *Aa*: $^1/_2$ *aa*
Uniform progeny	*AA* × *AA*	All *AA*
	aa × *aa*	All *aa*

	AA × *aa*	All *Aa*
	AA × *Aa*	All *A_*

The third phenotypic ratio is not really a ratio: all the offspring have the same phenotype (uniform progeny). Several combinations of parental genotypes can produce this outcome (see Table 3.5). A cross between any two homozygous parents—either between two parents of the same homozygous genotype (*AA* × *AA* or *aa* × *aa*) or between two parents with different homozygous genotypes (*AA* × *aa*)—produces progeny all having the same phenotype. Progeny of a single phenotype can also result from a cross between a homozygous dominant parent and a heterozygote (*AA* × *Aa*).

If we are interested in the ratios of genotypes instead of phenotypes, there are still only three outcomes to remember (**Table 3.6**): the 1:2:1 ratio, produced by a cross between two heterozygotes; the 1:1 ratio, produced by a cross between a heterozygote and a homozygote; and uniform progeny, produced by a cross between two homozygotes. These simple phenotypic and genotypic ratios, and the parental genotypes that produce them, provide the key to understanding crosses not only for a single locus but also, as you will see in the next section, for multiple loci.

TABLE 3.6 Genotypic ratios for simple genetic crosses (crosses for a single locus)

Genotypic Ratio	Genotypes of Parents	Genotypes of Progeny
1:2:1	*Aa* × *Aa*	$^1/_4$ *AA*: $^1/_2$ *Aa*: $^1/_4$ *aa*
1:1	*Aa* × *aa*	$^1/_2$ *Aa*:$^1/_2$ *aa*
	Aa × *AA*	$^1/_2$ *Aa*:$^1/_2$ *AA*
Uniform progeny	*AA* × *AA*	All *AA*
	aa × *aa*	All *aa*
	AA × *aa*	All *Aa*

3.3 Dihybrid Crosses Reveal the Principle of Independent Assortment

We now extend Mendel's principle of segregation to some more complex crosses that include alleles at multiple loci. Understanding the nature of these crosses will require an additional principle: the principle of independent assortment.

Dihybrid Crosses

In addition to his work on monohybrid crosses, Mendel crossed varieties of peas that differed in *two* characteristics—that is, he performed **dihybrid crosses**. For example, he crossed one homozygous variety that had seeds that were round and yellow with another homozygous variety that had seeds that were wrinkled and green. The seeds of all the F_1 progeny were round and yellow. He then allowed the F_1 to self-fertilize and obtained the following progeny in the F_2: 315 round, yellow seeds; 101 wrinkled, yellow seeds; 108 round, green seeds; and 32 wrinkled, green seeds. Mendel recognized that these traits appeared in a ratio of approximately 9 : 3 : 3 : 1; that is, $^{9}/_{16}$ of the progeny were round and yellow, $^{3}/_{16}$ were wrinkled and yellow, $^{3}/_{16}$ were round and green, and $^{1}/_{16}$ were wrinkled and green.

The Principle of Independent Assortment

Mendel carried out a number of dihybrid crosses for pairs of characteristics and always obtained a 9:3:3:1 ratio in the F_2. This ratio makes perfect sense in regard to the principle of segregation and the concept of dominance if we add a third principle, which Mendel recognized in his dihybrid crosses: the **principle of independent assortment** (Mendel's second law). This principle states that alleles at different loci separate independently of one another (see Table 3.2).

A common mistake is to think that the principle of segregation and the principle of independent assortment refer to two different processes. The principle of independent assortment is really an extension of the principle of segregation. The principle of segregation states that the two alleles at a locus separate when gametes are formed; the principle of independent assortment states that when these two alleles separate, their separation is independent of the separation of alleles at *other* loci.

Let's see how the principle of independent assortment explains the results that Mendel obtained in the dihybrid cross previously described. Each pea plant possesses two alleles encoding each characteristic, so the parent plants must have had genotypes *RR YY* and *rr yy* (**Figure 3.11a**). The principle of segregation tells us that the

alleles at each locus separate, and that one allele for each locus passes into each gamete. The gametes produced by the round, yellow parent therefore contained alleles *RY*, whereas the gametes produced by the wrinkled, green parent contained alleles *ry*. These two types of gametes united to produce the F_1, all with genotype *Rr Yy*. Because round is dominant to wrinkled and yellow is dominant to green, the phenotype of the F_1 was round and yellow.

Experiment

Question: Do alleles encoding different traits separate independently?

Methods

(a)

P generation

Round, yellow seeds × Wrinkled, green seeds

RR YY × *rr yy*

Gametes: *RY* × *ry*

Fertilization

(b)

F_1 generation

Round, yellow seeds

Rr Yy

Gametes: *RY*, *ry*, *Ry*, *rY*

Self-fertilization

Results

(c)

F_2 generation

	RY	*ry*	*Ry*	*rY*
RY	*RR YY*	*Rr Yy*	*RR Yy*	*Rr YY*
ry	*Rr Yy*	*rr yy*	*Rr yy*	*rr Yy*
Ry	*RR Yy*	*Rr yy*	*RR yy*	*Rr Yy*
rY	*Rr YY*	*rr Yy*	*Rr Yy*	*rr YY*

Phenotypic ratio
9 round, yellow : 3 round, green :
3 wrinkled, yellow : 1 wrinkled, green

Conclusion: The allele encoding color separated independently of the allele encoding seed shape, producing a 9 : 3 : 3 : 1 ratio in the F_2 progeny.

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3.11 Mendel's dihybrid crosses revealed the principle of independent assortment.

Description

The methods involve Part "a" on top with P generation. It shows round yellow seeds with genotype big R big R big Y big Y crossed with green wrinkled seeds with genotype small R small R small Y small Y. Gametes produced are big R big Y and small R small Y respectively. Fertilization takes place and leads to part "b" below which represents F subscript 1 generation, which consists of round yellow seeds with genotype big R small R big Y small Y. Four types of gametes are produced as follows: big R big Y, small R small Y, big R small Y, and small R big Y. Self-fertilization takes place and leads to part "c" below. Part c shows results through F subscript 2 generation. The results of the cross are represented with a Punnett square of 4 rows and 4 columns, each headed with gametes.

Row 1 column 1: an image shows a round yellow seed with genotype big R big R big Y big Y.

Row 1 column 2: an image shows a round yellow seed with genotype big R small R big Y small Y.

Row 1 column 3: an image shows a round yellow seed with genotype big R big R big Y small Y.

Row 1 column 4: an image shows a round yellow seed with genotype big R small R big Y big Y.

Row 2 column 1: an image shows a round yellow seed with genotype big R small R big Y small Y.

Row 2 column 2: an image shows a wrinkled green seed with genotype small R small R small Y small Y.

Row 2 column 3: an image shows a round green seed with genotype big R small R small Y small Y.

Row 2 column 4: an image shows a wrinkled yellow seed with genotype small R small R big Y small Y.

Row 3 column 1: an image shows a round yellow seed with genotype big R big R big Y small Y.

Row 3 column 2: an image shows a round green seed with genotype big R small R small Y small Y.

Row 3 column 3: an image shows a round green seed with genotype big R big R small Y small Y.

Row 3 column 4: an image shows a round yellow seed with genotype big R small R big Y small Y.

Row 4 column 1: an image shows a round yellow seed with genotype big R small R big Y big Y.

Row 4 column 2: an image shows a wrinkled yellow seed with genotype small R small R big Y small Y.

Row 4 column 3: an image shows a round yellow seed with genotype big R small R big Y small Y.

Row 4 column 4: an image shows a wrinkled yellow seed with genotype small R small R big Y big Y.

A caption below reads, “phenotypic ratio: 9 round, yellow to 3 round, green to 3 wrinkled, yellow to 1 wrinkled green.”

The conclusion states that the allele encoding color separated independently of the allele encoding seed shape, producing a 9 to 3 to 3 to 1 ratio in the F subscript 2 progeny.

When Mendel allowed the F_1 plants to self-fertilize to produce the F_2, the alleles at each locus separated, and one of those alleles

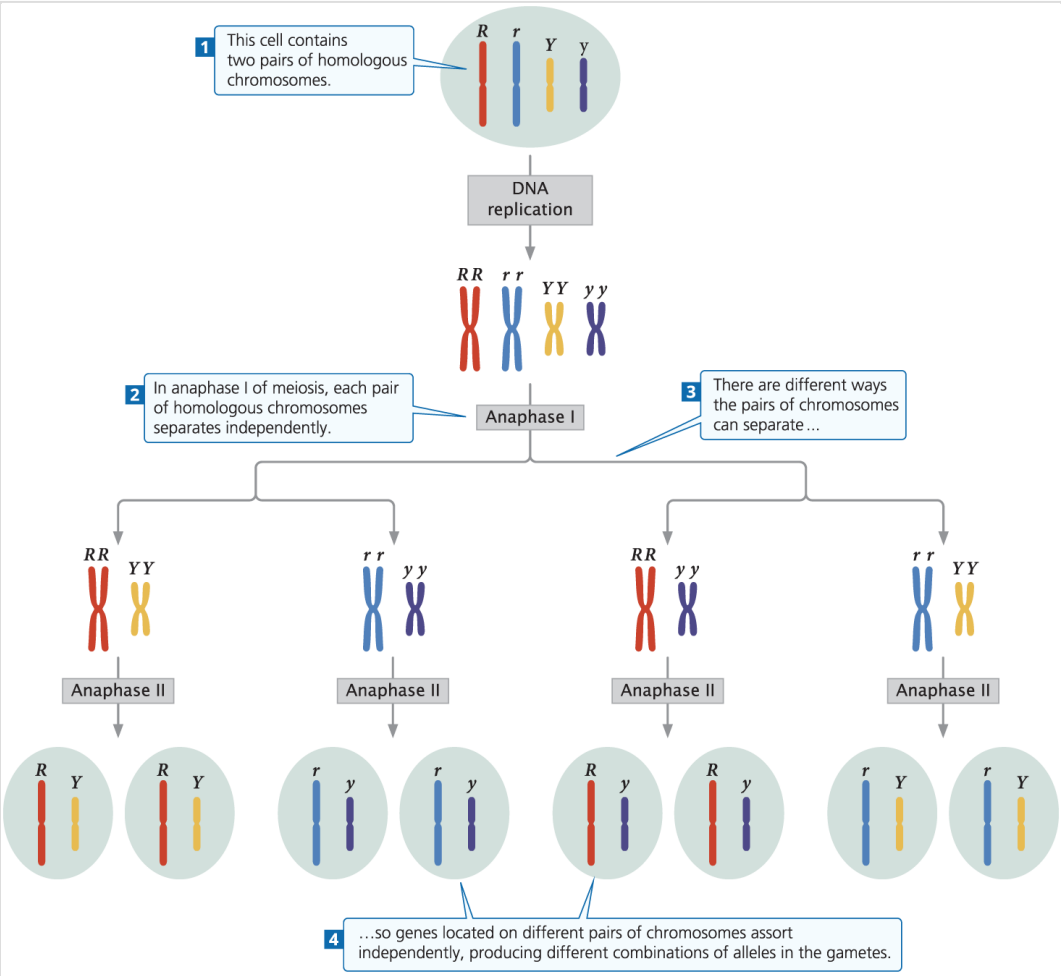
passed into each gamete. This event is where the principle of independent assortment becomes important. The two pairs of alleles can separate in two ways: (1) *R* separates with *Y*, and *r* separates with *y*, to produce gametes *RY* and *ry*, or (2) *R* separates with *y*, and *r* separates with *Y*, to produce gametes *Ry* and *rY*. The principle of independent assortment tells us that the alleles at each locus separate independently; thus, both kinds of separation take place with equal frequency, and all four types of gametes (*RY*, *ry*, *Ry*, and *rY*) are produced in equal proportions (Figure 3.11b). When these four types of gametes are combined to produce the F_2 generation, the progeny consist of $^{9}/_{16}$ round and yellow, $^{3}/_{16}$ round and green, $^{3}/_{16}$ wrinkled and yellow, and $^{1}/_{16}$ wrinkled and green, resulting in a 9:3:3:1 phenotypic ratio (Figure 3.11c).

THINK-PAIR-SHARE Question 6

Relating the Principle of Independent Assortment to Meiosis

An important qualification of the principle of independent assortment is that it applies to characteristics encoded by loci located on different chromosomes. Like the principle of segregation, it is based wholly on the behavior of chromosomes in meiosis. Each pair of homologous chromosomes separates independently of all

other pairs in anaphase I of meiosis (Figure 3.12), so genes located on different pairs of homologs will assort independently. Genes that happen to be located on the same chromosome will travel together during anaphase I of meiosis and will arrive at the same destination—within the same gamete (unless crossing over takes place). So genes located on the same chromosome do not assort independently (unless they are located sufficiently far apart that crossing over takes place in every meiotic division, a situation that will be discussed fully in Chapter 7).



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3.12 The principle of independent assortment results from the independent separation of chromosomes in anaphase I of meiosis.

Description

The illustration begins with a cell that contains two pairs of homologous chromosomes as follows: red large rod shaped chromosome big R, blue large rod shaped chromosome small R, yellow short rod shape d chromosome big Y, and purple short rod shaped chromosome small Y. A caption reads, this cell contains two pairs of homologous chromosomes. The cell undergoes chromosome replication and yields 4 different X shaped chromosomes with the following genotypes: red large X shaped chromosome with genotype big R big R, blue large X shaped chromosome with genotype small R small R, yellow short X shaped chromosome with genotype big Y big Y, and purple short X shaped chromosome with genotype small Y small Y. These chromosomes separate independently during anaphase 1. A caption reads, in anaphase 1 of meiosis, each pair of homologous chromosomes separates independently. There are different ways the pairs of chromosomes can separate.

The different ways these chromosomes can separate are: red large X shaped chromosome with genotype big R big R and yellow short X shaped chromosome with genotype big Y big Y; blue large X shaped chromosome with genotype small R small R and purple short X shaped chromosome with genotype small Y small Y on a branch. Red large X shaped chromosome with genotype big R big R and purple short X shaped chromosome with genotype small Y small Y; blue large X shaped chromosome with genotype small R small R and yellow short X shaped chromosome with genotype big Y big Y on the other branch. All these chromosome pairs undergo anaphase 2 and genes located on different pairs of chromosomes assort independently, producing different combinations of alleles in the gametes. The following gametes are produced:

Red large X shaped chromosome with genotype big R big R and yellow short X shaped chromosome with genotype big Y big Y produce two gametes with red rod shaped chromosome with genotype big R and yellow short rod shaped chromosome with genotype big Y each.

Blue large X shaped chromosome with genotype small R small R and purple short X shaped chromosome with genotype small Y small Y produce two gametes with blue large

rod shaped chromosome with genotype small R and purple short rod shaped chromosome with genotype small Y each.

Red large X shaped chromosome with genotype big R big R and purple short X shaped chromosome with genotype small Y small Y produce two gametes with red large rod shaped chromosome with genotype big R and purple short rod shaped chromosome with genotype small Y each.

Blue large X shaped chromosome with genotype small R small R and yellow short X shaped chromosome with genotype big Y big Y produce two gametes with blue large rod shaped chromosome with genotype small R and yellow short rod shaped chromosome with genotype big Y each.

A caption reads, so genes located on different pairs of chromosomes assort independently, producing different combinations of alleles in the gametes.

CONCEPTS

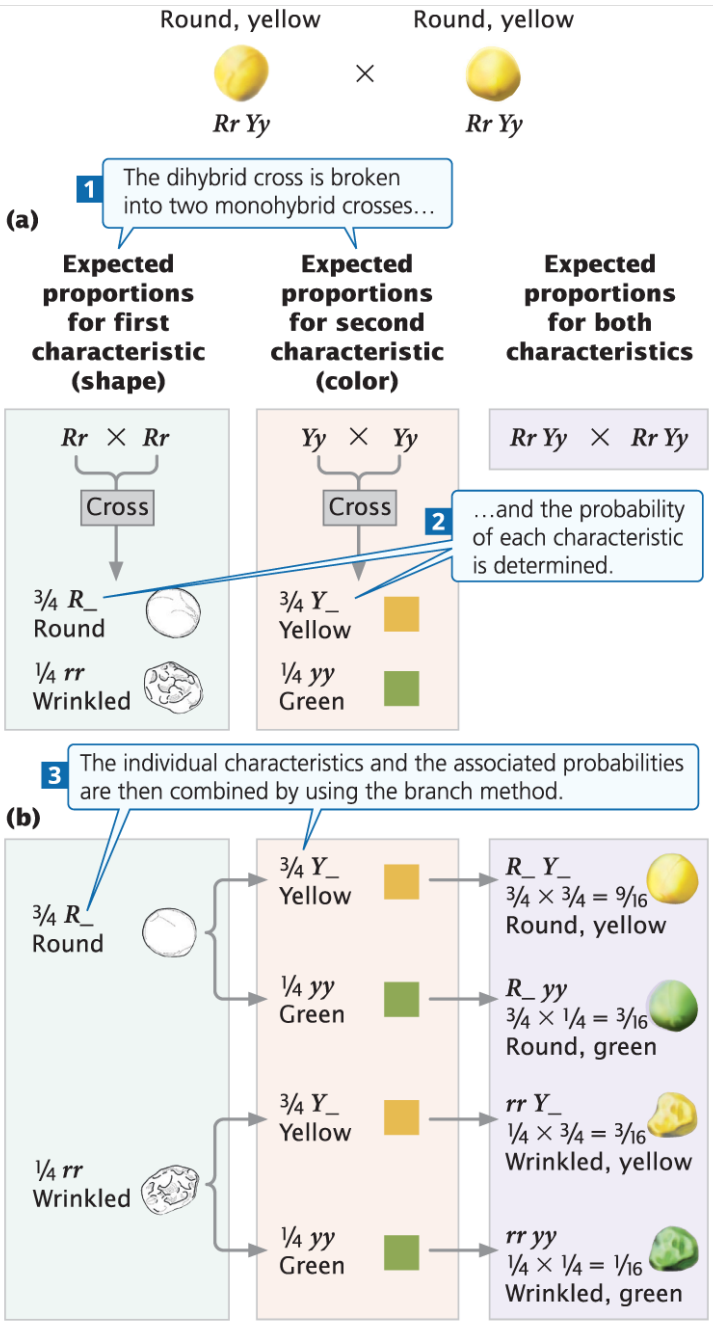
The principle of independent assortment states that genes encoding different characteristics separate independently of one another when gametes are formed, owing to the independent separation of homologous pairs of chromosomes in meiosis. Genes located close together on the same chromosome do not, however, assort independently.

✔ CONCEPT CHECK 6

How are the principles of segregation and independent assortment related, and how are they different?

Applying Probability and the Branch Diagram to Dihybrid Crosses

When the genes at two loci separate independently, a dihybrid cross can be understood as two monohybrid crosses. Let's examine Mendel's dihybrid cross (*Rr Yy* × *Rr Yy*) by considering each characteristic separately (**Figure 3.13a**). If we consider only the shape of the seeds, the cross was *Rr* × *Rr*, which yields a 3:1 phenotypic ratio ($^3/_4$ round and $^1/_4$ wrinkled progeny; see Table 3.5). Next, we consider the other characteristic, the color of the seeds. The cross was *Yy* × *Yy*, which produces a 3:1 phenotypic ratio ($^3/_4$ yellow and $^1/_4$ green progeny).



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3.13 A branch diagram can be used to determine the phenotypes and expected proportions of offspring from a dihybrid cross (*Rr Yy* × *Rr Yy*).

Description

The dibyhrid cross is a self-cross between round yellow seeds with genotype big R small R big Y small Y. Part “a” on top shows three distinct panels. First is for the expected proportions of the first characteristic (shape), second is for the expected proportions of the second characteristic (color), and third is for the expected proportions of both characteristics. A caption reads, “The dihybrid cross is broken into two monohybrid crosses.” The first panel on the left shows a self-cross between big R small R, which produces three-fourth round with genotype big R blank and one-fourth wrinkled with genotype small R small R. The second panel in the center shows a self-cross between big Y small Y, which produces three-fourth yellow with genotype big Y blank and one-fourth green with genotype small Y small Y. The third panel on the right shows a self-cross between big R small R big Y small Y. A caption extending out from three-fourth round and three-fourth yellow reads, “and the probability of each characteristic is determined.” Part “b” below shows a horizontal branch diagram and a caption reads, “The individual characters and the associated probabilities are then combined by using the branch method.” The branch diagram has two different branches for round seeds and wrinkled seeds.

First branch: Three-fourth big R blank, round branches into two sub-branches. The first sub-branch: three-fourth big Y blank, yellow which gives big R blank big Y blank (round yellow), three-fourth multiplied by three-fourth equals 9 over 16. The second sub-branch: one-fourth small Y small Y, green which gives big R blank small Y small Y (round green), three-fourth multiplied by one-fourth equals 3 over 16.

Second branch: One-fourth small R small R, wrinkled also branches into two sub-branches. The first sub-branch: three-fourth big Y blank, yellow which gives small R small R big Y blank (wrinkled yellow), one-fourth multiplied by three-fourth equals 3 over 16. The second sub-branch: one-fourth small Y small Y, green which gives small R small R small Y small Y, (wrinkled green), one-fourth multiplied by one-fourth equals 1 over 16.

We can now combine these monohybrid ratios by using the multiplication rule to obtain the proportion of progeny with different combinations of seed shape and color. The proportion of progeny with round and yellow seeds is $^{3}/_{4}$ (the probability of round) $\times$ $^{3}/_{4}$ (the probability of yellow) = $^{9}/_{16}$. The proportion of progeny with

round and green seeds is $^3/_4 \times {}^1/_4 = {}^3/_{16}$; the proportion of progeny with wrinkled and yellow seeds is $^1/_4 \times {}^3/_4 = {}^3/_{16}$; and the proportion of progeny with wrinkled and green seeds is $^1/_4 \times {}^1/_4 = {}^1/_{16}$.

Branch diagrams are a convenient way of organizing all the combinations of characteristics in the progeny of a cross (**Figure 3.13b**). In the first column, list the proportions of the phenotypes for one characteristic (here, seed shape: $^3/_4$ round and $^1/_4$ wrinkled). In the second column, list the proportions of the phenotypes for the next characteristic (seed color: $^3/_4$ yellow and $^1/_4$ green) twice, next to each of the phenotypes in the first column: write "$^3/_4$ yellow" and "$^1/_4$ green" next to the round phenotype and again next to the wrinkled phenotype. Check to make sure that the proportions for each characteristic sum to one (i.e., $^3/_4$ yellow and $^1/_4$ green = 1.0). Now, draw lines between the phenotypes in the first column and each of the phenotypes in the second column. Follow each branch of the diagram, multiplying the probabilities for each trait along that branch. One branch leads from round to yellow, yielding round and yellow progeny. Another branch leads from round to green, yielding round and green progeny, and so forth. We calculate the probability of progeny with a particular combination of traits by using the multiplication rule: the probability of round ($^3/_4$) and yellow ($^3/_4$) seeds is $^3/_4 \times {}^3/_4 = {}^9/_{16}$. The advantage of the branch diagram is that it helps us keep track of all the potential combinations of traits that may appear in the progeny. It can be used to determine phenotypic or genotypic ratios for any number of characteristics.

Using probability is much faster than using the Punnett square for crosses that include multiple loci. Genotypic and phenotypic ratios can be quickly worked out by combining, using the multiplication rule, the simple ratios in Tables 3.5 and 3.6. The probability method is particularly efficient if we need the probability of only a *particular* phenotype or genotype among the progeny of a cross. Suppose that we need to know the probability of obtaining the genotype *Rr yy* in the F_2 of the dihybrid cross in Figure 3.11. The probability of obtaining the *Rr* genotype in a cross of *Rr* × *Rr* is $^1/_2$, and that of obtaining the *yy* genotype in a cross of *Yy* × *Yy* is $^1/_4$ (see Table 3.6). Using the multiplication rule, we find the probability of *Rr yy* to be $^1/_2 \times {}^1/_4 = {}^1/_8$.

To illustrate the advantage of the probability method, consider the cross *Aa Bb cc Dd Ee* × *Aa Bb Cc dd Ee*. Suppose that we want to know the probability of obtaining offspring with the genotype *aa bb cc dd ee*. If we use a Punnett square to determine this probability, we might be working on the solution for months. However, we can quickly figure the probability of obtaining this one genotype by breaking this cross into a series of single-locus crosses:

Progeny cross	Genotype	Probability
Aa × *Aa*	*aa*	$^1/_4$
Bb × *Bb*	*bb*	$^1/_4$

cc × *Cc*	*cc*	$^1/_2$
Dd × *dd*	*dd*	$^1/_2$
Ee × *Ee*	*ee*	$^1/_4$

The probability of an offspring from this cross having genotype *aa bb cc dd ee* is now easily obtained by using the multiplication rule: $^1/_4 \times {}^1/_4 \times {}^1/_2 \times {}^1/_2 \times {}^1/_4 = {}^1/_{256}$. This calculation assumes that the genes at these five loci all assort independently.

Now that you've had some experience working genetic crosses, explore Mendel's principles of heredity by setting up some of your own crosses in (A) **Animation 3.1**.

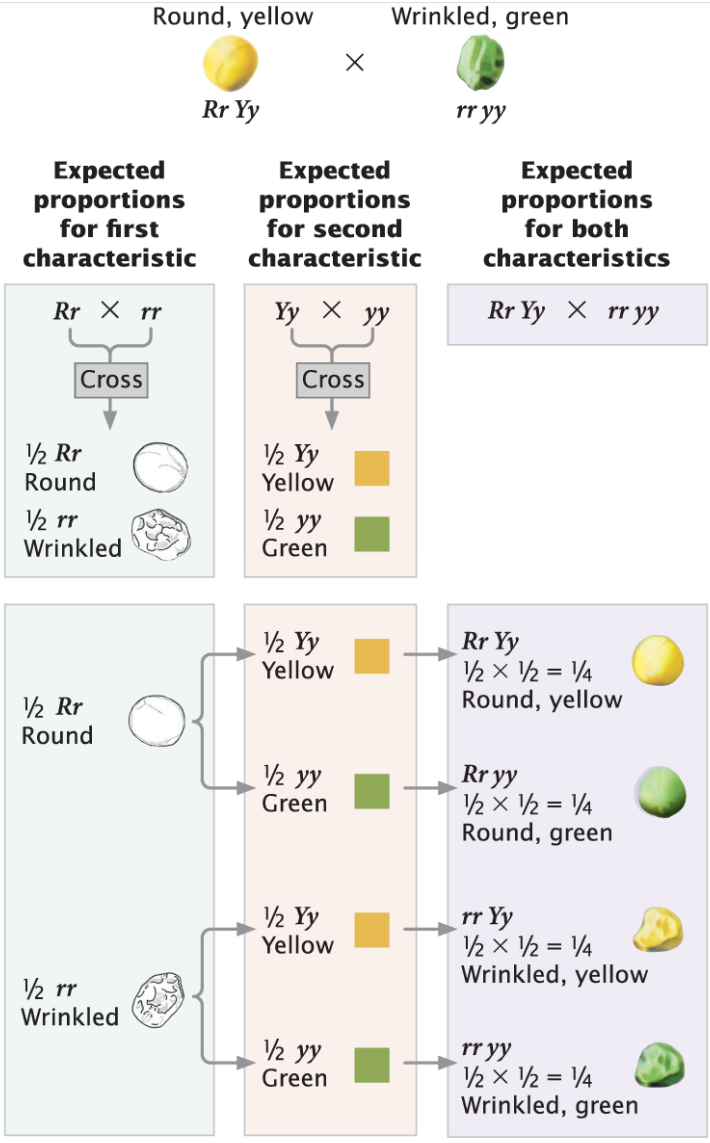
CONCEPTS

A cross including several characteristics can be worked by breaking it down into single-locus crosses and using the multiplication rule to determine the proportions of combinations of characteristics (provided that the genes assort independently).

The Dihybrid Testcross

Let's practice using the branch diagram by determining the types and proportions of phenotypes in a dihybrid testcross between the

round and yellow F_1 pea plants (*Rr Yy*) obtained by Mendel in his dihybrid cross and the wrinkled and green pea plants (*rr yy*), as depicted in **Figure 3.14**. First, break the cross down into a series of single-locus crosses. The cross *Rr* × *rr* yields $^1/_2$ round (*Rr*) progeny and $^1/_2$ wrinkled (*rr*) progeny. The cross *Yy* × *yy* yields $^1/_2$ yellow (*Yy*) progeny and $^1/_2$ green (*yy*) progeny. Using the multiplication rule, we find the proportion of round and yellow progeny to be $^1/_2$ (the probability of round) × $^1/_2$ (the probability of yellow) = $^1/_4$. Four combinations of traits appear in the offspring in the following proportions: $^1/_4$ *Rr Yy*, round and yellow; $^1/_4$ *Rr yy*, round and green; $^1/_4$ *rr Yy*, wrinkled and yellow; and $^1/_4$ *rr yy*, wrinkled and green.



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3.14 A branch diagram can be used to determine the phenotypes and expected proportions of offspring from a dihybrid testcross (*Rr Yy* × *rr yy*).

Description

The dibyhrid cross is between round yellow seeds with genotype big R small R big Y small Y and wrinkled green seeds with genotype small R small R small Y small Y. The illustration shows three distinct panels. First is for the expected proportions of the first characteristic, second is for the expected proportions of the second characteristic, and third is for the expected proportions of both characteristics. The first panel on the left shows a cross

between big R small R and small R small R, which produces one-half round with genotype big R small R and one-half wrinkled with genotype small R small R. The second panel in the center shows a cross between big Y small Y and small Y small Y, which produces one-half yellow with genotype big Y small Y and one-fourth green with genotype small Y small Y. The third panel on the right shows a cross between big R small R big Y small Y and small R small R small Y small Y. Below is a horizontal branch diagram. The branch diagram has two different branches for round seeds and wrinkled seeds.

First branch: one-half big R small R, round branches into two sub-branches. The first sub-branch: one-half big Y small Y, yellow which gives big R small R big Y small Y (round yellow), one-half multiplied by one-half equals one-fourth. The second sub-branch: one-half small Y small Y, green which gives big R small R small Y small Y (round green), one-half multiplied by one-half equals one-fourth.

Second branch: One-half small R small R, wrinkled also branches into two sub-branches. The first sub-branch: one-half big Y small Y, yellow which gives small R small R big Y small Y (wrinkled yellow), one-half multiplied by one-half equals one-fourth. The second sub-branch: one-half small Y small Y, green which gives small R small R small Y small Y, (wrinkled green), one-half multiplied by one-half equals one-fourth.

THINK-PAIR-SHARE Question 7

WORKED PROBLEM

The principles of segregation and independent assortment are important not only because they explain how heredity works but also because they provide the means for predicting the outcome of genetic crosses. This predictive power has made genetics a powerful tool in agriculture and other fields, and the ability to apply the principles of heredity is an important skill for all students of

genetics. Practice with genetic problems is essential for mastering the basic principles of heredity; no amount of reading and memorization can substitute for the experience gained by deriving solutions to specific problems in genetics.

You may find genetics problems difficult if you are unsure of where to begin or how to organize a solution to the problem. In genetics, every problem is different, so no common series of steps can be applied to all genetics problems. Logic and common sense must be used to analyze a problem and arrive at a solution. Nevertheless, certain steps can facilitate the process, and solving the following problem will serve to illustrate those steps.

In mice, black coat color (*B*) is dominant to brown (*b*), and a solid pattern (*S*) is dominant to a white-spotted pattern (*s*). Color and spotting are controlled by genes that assort independently. A homozygous black, spotted mouse is crossed with a homozygous brown, solid mouse. All the F_1 mice are black and solid. A testcross is then carried out by mating the F_1 mice with brown, spotted mice.

a. Give the genotypes of the parents and the F_1 mice.
b. Give the genotypes and phenotypes, along with their expected ratios, for the progeny expected from the testcross.

Solution Strategy

What information is required in your answer to the problem?

First, determine what question or questions the problem is asking. Is it asking for genotypes or genotypic ratios or phenotypic ratios? This problem asks you to provide the *genotypes* of the parents and the F_1, the *expected genotypes* and *phenotypes* of the progeny of the testcross, and their *expected proportions*.

What information is provided to solve the problem?

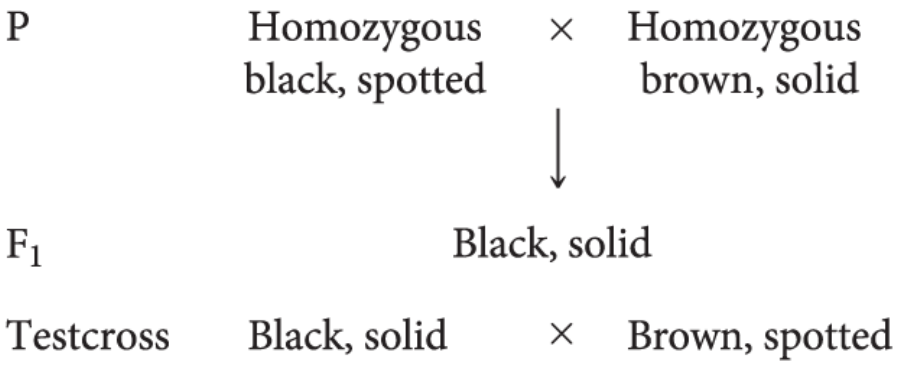
Next, determine what information is provided that will be necessary to solve the problem. This problem gives important information about the dominance relations of the traits involved and the genes that encode them:

- Black is dominant to brown.
- Solid is dominant to spotted.
- The genes for the two characteristics assort independently.
- Symbols for the different alleles: *B* for black, *b* for brown, *S* for solid, and *s* for spotted.

It is often helpful to write down the symbols at the beginning of the solution:

B—black	*S*—solid
b—brown	*s*—spotted

Next, write out the crosses given in the problem:



Description
In the parent generation labeled as P, homozygous black, spotted with genotype big B big B small S small S is crossed with homozygous brown, solid with genotype small B small B big S big S. A downward arrow points to black, solid in the F subscript 1 generation with genotype big B blank big S blank. Below F subscript 1 generation, a test cross is shown between black, solid with genotype big B blank big S blank and black spotted with genotype small B small B small S small S.

For help with this problem, review:

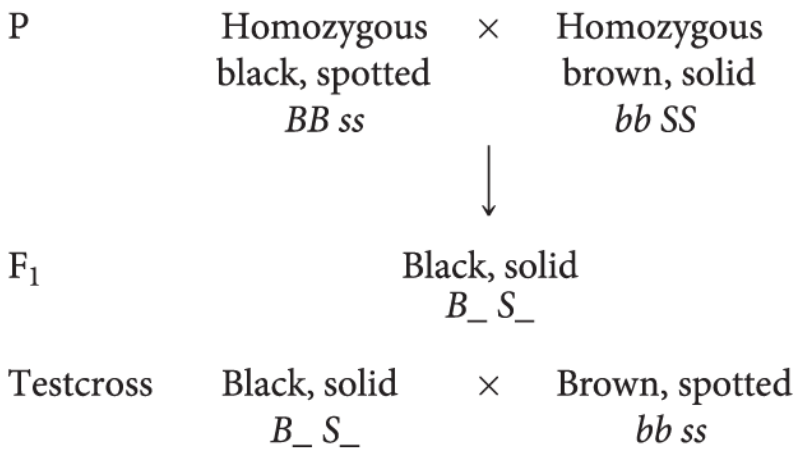
If you need help solving the problem, review those sections of the chapter that cover the relevant information. For this problem, review Sections 3.2 and 3.3.

Solution Steps

STEP 1 Write down any genetic information that can be determined from the phenotypes alone.

From their phenotypes and the statement that they are homozygous, you know that the P-generation mice must be *BB ss* and *bb SS*. The F_1 mice are black and solid, both of which are dominant traits, so the

F_1 mice must possess at least one black allele (*B*) and one solid allele (*S*). At this point, you may not be certain about the other alleles, so you can represent the genotype of the F_1 as *B_ S_*, where _ means that any allele is possible. The brown, spotted mice used in the testcross must be *bb ss* because both brown and spotted are recessive traits that will be expressed only if two recessive alleles are present. Record these genotypes on the crosses that you wrote out:



Description
In parent generation, homozygous black spotted is crossed with homozygous brown solid. A downward arrow points to black, solid in the F subscript 1 generation. A test cross is shown below between black solid and black spotted.

STEP 2 Break the problem down into smaller parts.

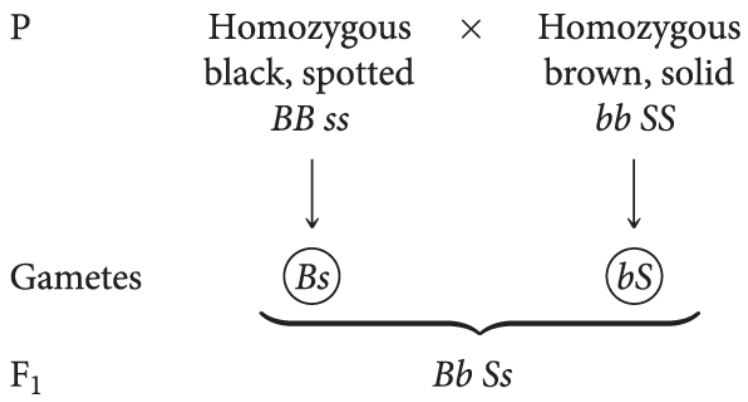
First, determine the genotype of the F_1. After this genotype has been determined, you can predict the results of the testcross and determine the genotypes and phenotypes of the progeny of the testcross.

Second, because this cross includes two independently assorting loci, it can be conveniently broken down into two single-locus crosses: one for coat color and the other for spotting.

Third, you can use a branch diagram to determine the proportion of progeny of the testcross with different combinations of the two traits.

STEP 3 Work the different parts of the problem.

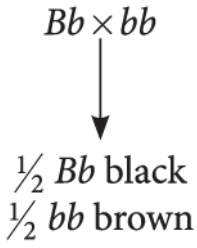
Start by determining the genotype of the F_1 progeny. Mendel's first law indicates that the two alleles at a locus separate, one going into each gamete. Thus, the gametes produced by the black, spotted parent contain *B s* and the gametes produced by the brown, solid parent contain *b S*, which combine to produce F_1 progeny with the genotype *Bb Ss*:



Description
In the parent generation, homozygous black, spotted with genotype big B big B small S small S is crossed with homozygous brown, solid with genotype small B small B big S big

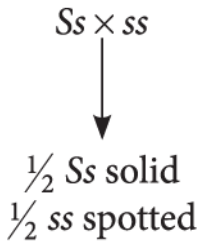
S. Gametes produced are big B small S and small B big S respectively. These gametes produce the F subscript 1 generation with genotype big B small B big S small S.

Use the F_1 genotype to work the testcross (*Bb Ss* × *bb ss*), breaking it into two single-locus crosses. First, consider the cross for coat color: *Bb* × *bb*. Any cross between a heterozygote and a homozygous recessive genotype produces a 1:1 phenotypic ratio of progeny (see Table 3.5):



Description
Big B small B is crossed with small B small B. It yields one-half big B small B, black and one-half small B small B, brown.

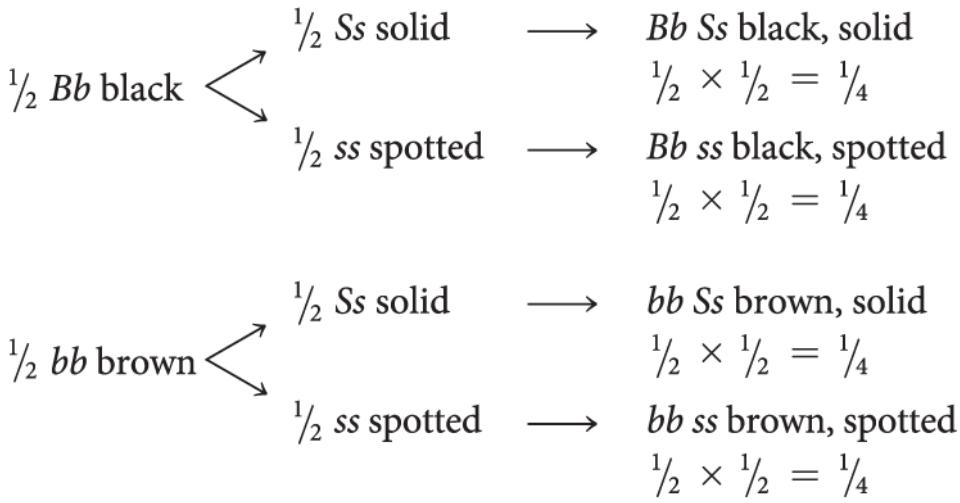
Next, consider the cross for spotting: *Ss* × *ss*. This cross is also between a heterozygote and a homozygous recessive genotype and produces $^1/_2$ solid (*Ss*) and $^1/_2$ spotted (*ss*) progeny (see Table 3.5).



Description

Big S small S is crossed with small S small S. It yields one-half big S small S, solid and one-half small S small S, spotted.

Finally, determine the proportions of progeny with combinations of these characters by using a branch diagram.



Description
The branch diagram has two different branches for black mice and brown mice.

First branch: one-half big B small B, black branches into two sub-branches. The first sub-branch: one-half big S small S, solid which gives big B small B big S small S (black solid), one-half multiplied by one-half equals one-fourth. The second sub-branch: one-half small S small S, spotted which gives big B small B small S small S (black spotted), one-half multiplied by one-half equals one-fourth.

Second branch: One-half small B small B, brown also branches into two sub-branches. The first sub-branch: one-half big S small S, solid which gives small B small B big S small S (brown solid), one-half multiplied by one-half equals one-fourth. The second sub-branch: one-half small S small S, spotted which gives small B small B small S small S, (brown spotted), one-half multiplied by one-half equals one-fourth.

STEP 4 Check all work.

As a last step, reread the problem, checking to see if your answers are consistent with the information provided. You have used the genotypes *BB ss* and *bb SS* in the P generation. Do these genotypes encode the phenotypes given in the problem? Are the F_1 progeny phenotypes consistent with the genotypes that you assigned? The answers are consistent with the information.

» Now that we have stepped through a genetics problem together, try your hand at **Problem 33** at the end of this chapter.

3.4 Observed Ratios of Progeny May Deviate from Expected Ratios by Chance

When two individual organisms of known genotype are crossed, we expect certain ratios of genotypes and phenotypes among the progeny; these expected ratios are based on the Mendelian principles of segregation, independent assortment, and dominance. The ratios of genotypes and phenotypes *actually* observed among the progeny, however, may deviate from these expectations.

For example, in German cockroaches, brown body color (*Y*) is dominant to yellow body color (*y*). If we cross a brown, heterozygous cockroach (*Yy*) with a yellow cockroach (*yy*), we expect a 1:1 ratio of brown (*Yy*) and yellow (*yy*) progeny. Among 40 progeny, we therefore expect to see 20 brown and 20 yellow offspring. However, the observed numbers might deviate from these expected values; we might in fact see 22 brown and 18 yellow progeny.

Chance plays a critical role in genetic crosses, just as it does in flipping a coin. When you flip a coin, you expect a 1:1 ratio—$^1/_2$ heads and $^1/_2$ tails. If you flipped a coin 1000 times, the proportion of heads and tails obtained would probably be very close to that expected 1:1 ratio. However, if you flipped the coin 10 times, the ratio of heads to tails might be quite different from 1:1. You could

easily get 6 heads and 4 tails, or 3 heads and 7 tails, just by chance. You might even get 10 heads and 0 tails. The same thing happens in genetic crosses. We may expect 20 brown and 20 yellow cockroaches, but 22 brown and 18 yellow progeny *could* arise as a result of chance.

The Chi-Square (χ^2) Goodness-of-Fit Test

If you expected a 1:1 ratio of brown and yellow cockroaches but the cross produced 22 brown and 18 yellow cockroaches, you probably wouldn't be too surprised, even though it wasn't a perfect 1:1 ratio. In this case, it seems reasonable to assume that chance produced the deviation between the expected and the observed results. But if you observed 25 brown and 15 yellow cockroaches, would you still assume that this result represents a 1:1 ratio? Something other than chance might have caused this deviation. Perhaps the inheritance of this characteristic is more complicated than was assumed, or perhaps some of the yellow progeny died before they were counted. Clearly, we need some means of evaluating how likely it is that chance is responsible for a deviation between the observed and the expected numbers.

To evaluate the role of chance in producing deviations between observed and expected values, a statistical test called the **chi-square**

goodness-of-fit test is used. This test provides information about how well observed values fit expected values. Before we learn how to use this test, however, it is important to understand what it does and does not indicate about a genetic cross. The chi-square test cannot tell us whether a genetic cross has been correctly carried out, whether the results are correct, or whether we have chosen the correct genetic explanation for the results. What it does indicate is the *probability* that the difference between the observed and the expected values is due to chance. In other words, it indicates the likelihood that chance alone could produce the deviation between the expected and the observed values.

If we expected 20 brown and 20 yellow progeny from a genetic cross, the chi-square test gives us the probability that we might observe 25 brown and 15 yellow progeny simply owing to chance deviations from the expected 20:20 ratio. This hypothesis—that chance alone is responsible for a deviation between observed and expected values—is sometimes called the *null hypothesis*. Statistical tests such as the chi-square test cannot prove that the null hypothesis is correct, but they can help us decide whether we should reject it. When the probability calculated with the chi-square test is high, we assume that chance alone produced the deviation, and we do not reject the null hypothesis. When the probability is low, we assume that some factor other than chance—some significant factor—produced the deviation; for example, the mortality rate of the yellow cockroaches might be higher than that of the brown cockroaches. When the

probability that chance produced the deviation is low, we reject the null hypothesis.

To use the chi-square goodness-of-fit test, we first determine the expected results. The chi-square test must always be applied to *numbers* of progeny, not to proportions or percentages. Let's consider a locus for coat color in domestic cats, for which black color (*B*) is dominant to gray (*b*). If we crossed two heterozygous black cats (*Bb* × *Bb*), we would expect a 3:1 ratio of black and gray kittens. Imagine that a series of such crosses yields a total of 50 kittens—30 black and 20 gray. These numbers are our *observed* values. We can obtain the *expected* numbers by multiplying the expected proportions by the total number of observed progeny. In this case, the expected number of black kittens is $^3/_4 \times 50 = 37.5$, and the expected number of gray kittens is $^1/_4 \times 50 = 12.5$. Note that it is biologically impossible to have 37.5 or 12.5 kittens, because kittens only come in whole numbers, but the expected number is 37.5, so that is what we should use in the chi-square calculations.

The chi-square (χ^2) value is calculated by using the following formula:

$$\chi^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

where Σ means the sum. We calculate the sum of all the squared differences between observed and expected values divided by the

expected values. To calculate the chi-square value for our black and gray kittens, we first subtract the number of *expected* black kittens from the number of *observed* black kittens (30 − 37.5 = − 7.5) and square this value: $-7.5^2 = 56.25$. We then divide this result by the expected number of black kittens, 56.25/37.5 = 1.5. We repeat the calculations on the number of expected gray kittens: $(20 - 12.5)^2/12.5 = 4.5$. To obtain the overall chi-square value, we sum the (observed − expected)^2/expected values: 1.5 + 4.5 = 6.0.

The next step is to determine the probability associated with this calculated chi-square value, which is the probability that the deviation between the observed and the expected results is due to chance. This step requires us to compare our calculated chi-square value (6.0) with theoretical values in a chi-square table that have the same degrees of freedom. The degrees of freedom represent the number of ways in which the expected classes are free to vary. For a chi-square goodness-of-fit test, the degrees of freedom are equal to $n - 1$, in which n is the number of different expected phenotypes. Here, we lose one degree of freedom because the total number of expected progeny must equal the total number of observed progeny. In our example, there are two expected phenotypes (black and gray), so $n = 2$, and the degree of freedom equals 2 − 1 = 1.

Now that we have our calculated chi-square value and have figured out the associated degrees of freedom, we are ready to obtain the probability from a chi-square table (**Table 3.7**). The degrees of freedom are given in the left-hand column of the table and the

probabilities are given at the top; within the body of the table are chi-square values associated with these probabilities. First, we find the row for the appropriate degrees of freedom; for our example with 1 degree of freedom, it is the first row of the table. Then, we find our calculated chi-square value (6.0) among the theoretical values in this row. The theoretical chi-square values increase, and the probabilities decrease, from left to right. Our chi-square value of 6.0 falls between the value of 5.024, associated with a probability of 0.025, and the value of 6.635, associated with a probability of 0.01. Thus, the probability associated with our chi-square value is less than 0.025 and greater than 0.01. So there is less than a 2.5% probability that the deviation that we observed between the expected and the observed numbers of black and gray kittens could be due to chance.

TABLE 3.7 Critical values of the χ^2 distribution

	P								
df	**0.995**	**0.975**	**0.9**	**0.5**	**0.1**	**0.05***	**0.025**	**0.01**	**0.005**
1	0.000	0.000	0.016	0.455	2.706	3.841	5.024	6.635	7.879
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210	10.597
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345	12.838
4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277	14.860
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086	16.750
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812	18.548
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475	20.278
8	1.344	2.180	3.490	7.344	13.362	15.507	17.535	20.090	21.955
9	1.735	2.700	4.168	8.343	14.684	16.919	19.023	21.666	23.589

10	2.156	3.247	4.865	9.342	15.987	18.307	20.483	23.209	25.188
11	2.603	3.816	5.578	10.341	17.275	19.675	21.920	24.725	26.757
12	3.074	4.404	6.304	11.340	18.549	21.026	23.337	26.217	28.300
13	3.565	5.009	7.042	12.340	19.812	22.362	24.736	27.688	29.819
14	4.075	5.629	7.790	13.339	21.064	23.685	26.119	29.141	31.319
15	4.601	6.262	8.547	14.339	22.307	24.996	27.488	30.578	32.801

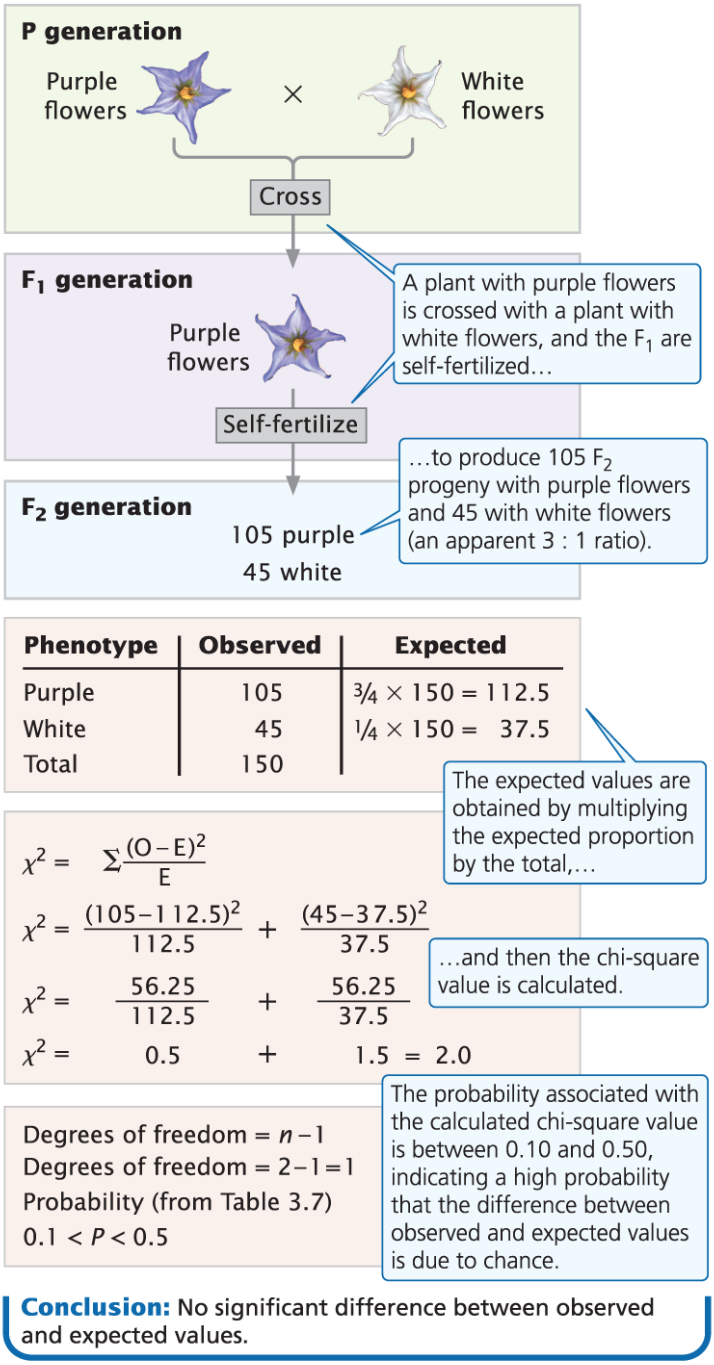
P, probability; *df*, degrees of freedom.

*Most scientists assume that when $P < 0.05$, a significant difference exists between the observed and the expected values in a chi-square test.

Most scientists use the 0.05 probability level as their cutoff value: if the probability of chance being responsible for the deviation between observed and expected values is greater than or equal to 0.05, they accept that chance may be responsible for the deviation. When the probability is less than 0.05, scientists assume that chance is not responsible and that a significant difference from the expected values exists. The expression *significant difference* means that a factor other than chance is responsible for the deviation between the observed and the expected values. In regard to the kittens, perhaps one of the genotypes had a greater mortality rate before the progeny were counted, or perhaps other genetic factors skewed the observed ratios.

In choosing 0.05 as the cutoff value, scientists have agreed to assume that chance is responsible for deviations between observed and expected values unless there is strong evidence to the contrary. Bear in mind that even if we obtain a probability of, say, 0.01, there is still a 1% probability that the deviation between the observed and the

expected values is due to nothing more than chance. Calculation of the chi-square value is illustrated in Figure 3.15. ❯ **TRY** PROBLEM 38



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3.15 A chi-square goodness-of-fit test is used to determine the probability that the difference between observed and expected values is due to chance.

Description

A cross between purple flowers and white flowers shows P through F subscript 2 generation. In P generation at the top, purple flowers are crossed with white flowers and the F subscript 1 generation has purple flowers only while the F subscript 2 generation has 105 purple and 45 white flowers. Drawings of purple and white flowers are shown. A caption extending out from the P generation Cross and the Self-fertilization F subscript 1 generation reads, “A plant with purple flowers is crossed with a plant with white flowers, and the F subscript 1 are self-fertilized to produce 105 F subscript 2 progeny with purple flowers and 45 with white flowers (an apparent 3 to 1 ratio).”

The observed and expected values for different phenotypes below are as follows:

Purple: observed, 105; expected, three-fourth multiplied by 150 equals 112 point 5.

White: observed, 45; expected, one-fourth multiplied by 150 equals 37 point 5.

Total: 150

A corresponding caption reads, “The expected values are obtained by multiplying the expected proportion by the total and then the chi-square value is calculated.” The calculation is as follows:

Chi-square equals summation start fraction left parenthesis O minus E right parenthesis squared over E end fraction. Chi-square equals start fraction left parenthesis 105 minus 112 point 5 right parenthesis squared over 112 point 5 end fraction plus start fraction left parenthesis 45 minus 37 point 5 right parenthesis squared over 37 point 5 end fraction. Chi-square equals start fraction 56 point 2 5 over 112 point 5 end fraction plus start fraction 56 point 2 5 over 37 point 5 end fraction. Chi square equals 0 point 5 plus 1 point 5 equals 2 point 0. Degrees of freedom equals n minus 1. Degrees of freedom equals 2 minus 1 equals 1. Probability (from table 3 point 7) 0 point 1 is less than P is less than 0 point 5. A corresponding caption reads, “The probability associated with the calculated chi-square value is between 0 point 10 and 0 point 50, indicating a high probability that the difference between observed and expected values is due to chance.”

The conclusion states that no significant difference between observed and expected values.

THINK-PAIR-SHARE Question 8

CONCEPTS

Differences between observed and expected ratios among the progeny of a cross can arise by chance. The chi-square goodness-of-fit test can be used to evaluate whether deviations between observed and expected numbers are likely to be due to chance or to some other, significant factor.

✔ CONCEPT CHECK 7

A chi-square test comparing observed and expected numbers of progeny is carried out, and the probability associated with the calculated chi-square value is 0.72. What does this probability represent?

a. Probability that the correct results were obtained
b. Probability of obtaining the observed numbers
c. Probability that the difference between observed and expected numbers is significant
d. Probability that the difference between observed and expected numbers could be due to chance

Chapter 3 Review

CHAPTER SUMMARY

Section 3.1 Learning Objective: Be able to explain how Gregor Mendel discovered the principles of heredity.

- Gregor Mendel discovered the principles of heredity. His success can be attributed to his choice of the pea plant as an experimental organism, the use of characteristics with a few easily distinguishable phenotypes, his experimental approach, the use of mathematics to interpret his results, and careful attention to detail.
- Genes are inherited factors that determine a characteristic. Alternative forms of a gene are called alleles. The alleles are located at a specific place, called a locus, on a chromosome, and the set of alleles that an individual organism possesses is its genotype. A phenotype is the manifestation or appearance of a characteristic and may refer to a physical, physiological, biochemical, or behavioral characteristic. Only the genotype—not the phenotype—is inherited.

Section 3.2 Learning Objective: Be able to use principles of heredity to predict progeny produced in a simple genetic cross.

- The principle of segregation states that a diploid individual organism possesses two alleles encoding a trait and that these two alleles separate in equal proportions when gametes are formed.
- The concept of dominance indicates that when two different alleles are present in a heterozygote, only the trait encoded by one of them, the dominant allele, is observed in the phenotype. The other allele is said to be recessive.
- The two alleles of a genotype are located on homologous chromosomes. The separation of homologous chromosomes in anaphase I of meiosis brings about the segregation of alleles.
- Probability is the likelihood that a particular event will occur. The multiplication rule states that the probability of two or more independent events occurring together is calculated by multiplying the probabilities of the independent events. The addition rule states that the probability of any of two or more mutually exclusive events occurring is calculated by adding the probabilities of the events.
- The binomial expansion can be used to determine the probability of a particular combination of events.
- A testcross, which reveals the genotype (homozygote or heterozygote) of an individual organism that has a dominant trait, consists of crossing that individual with one that has the homozygous recessive genotype.

Section 3.3 Learning Objective: Be able to predict the progeny of crosses involving two or more loci.

- The principle of independent assortment states that genes encoding different characteristics separate independently when gametes are formed. Independent assortment is based on the random separation of homologous pairs of chromosomes in anaphase I of meiosis; it takes place when genes encoding different characteristics are located on different pairs of chromosomes.

Section 3.4 Learning Objective: Be able to use the chi-square goodness-of-fit test to determine the probability that observed numbers of progeny differ from expected numbers.

- Observed ratios of progeny from a genetic cross may deviate from the expected ratios owing to chance. The chi-square goodness-of-fit test can be used to determine the probability that a difference between observed and expected numbers could be due to chance.

WORKED PROBLEMS

Problem 1

Short hair (*S*) in rabbits is dominant to long hair (*s*). The following crosses are carried out, producing the progeny shown. Give all possible genotypes of the parents in each cross.

	Parents	Progeny

a.	short × short	4 short and 2 long
b.	short × short	8 short
c.	short × long	12 short
d.	short × long	3 short and 1 long
e.	long × long	2 long

» Solution Strategy

What information is required in your answer to the problem?

All possible genotypes of the parents in each cross.

> **Note:** The problem asks for all possible genotypes of the parents.

What information is provided to solve the problem?

- Short hair is dominant to long hair.
- Phenotypes of the parents in each cross.
- Phenotypes and number of progeny of each cross.

For help with this problem, review:

Connecting Concepts: Ratios in Simple Crosses in Section 3.2.

» Solution Steps

For this problem, it is useful to first gather as much information about the genotypes of the parents as possible on the basis of their phenotypes. We can then look at the types of progeny produced to provide the missing information.

a. short $\times$ short $\rightarrow$ 4 short and 2 long

> **Note:** When both parents are heterozygotes (*Ss* × *Ss*), we expect to see a 3:1 ratio in progeny, but just by chance the progeny exhibited a 4:2 ratio.

Because short hair is dominant to long hair, a rabbit with short hair could be either *SS* or *Ss*. The two long-haired offspring must be homozygous (*ss*) because long hair is recessive and will appear in the phenotype only when two alleles for long hair are present. Because each parent contributes one of the two alleles found in the progeny, each parent must be carrying the *s* allele and must therefore be *Ss*.

b. short $\times$ short $\rightarrow$ 8 short

The short-haired parents could be *SS* or *Ss*. All eight of the offspring are short haired (S_), so at least one of the parents is likely to be homozygous (*SS*); if both parents were heterozygous, we would expect $^1/_4$ of the progeny to be long haired (*ss*), but we do not observe any long-haired progeny. The other parent could be homozygous (*SS*) or heterozygous (*Ss*); as long as one parent

is homozygous, all the offspring will be short haired. It is theoretically possible, although unlikely, that both parents are heterozygous (*Ss* × *Ss*). If both were heterozygous, we would expect two of the eight progeny to be long haired. Although no long-haired progeny are observed, it is possible that just by chance no long-haired rabbits were produced among the eight progeny of the cross.

c. short × long 12 short

The short-haired parent could be *SS* or *Ss*. The long-haired parent must be *ss*. If the short-haired parent were heterozygous (*Ss*), half of the offspring would be expected to be long haired, but we don't see any long-haired progeny. Therefore, this parent is most likely homozygous (*SS*). It is theoretically possible, although unlikely, that the parent is heterozygous and just by chance no long-haired progeny were produced.

d. short × long 3 short and 1 long

On the basis of its phenotype, the short-haired parent could be homozygous (*SS*) or heterozygous (*Ss*), but the presence of one long-haired offspring tells us that the short-haired parent must be heterozygous (*Ss*). The long-haired parent must be homozygous (*ss*).

e. long × long 2 long

Because long hair is recessive, both parents must be homozygous for a long-hair allele (*ss*).

Problem 2

In cats, black coat color is dominant to gray. A female black cat whose mother is gray mates with a gray male. If this female has a litter of six kittens, what is the probability that three will be black and three will be gray?

» Solution Strategy

What information is required in your answer to the problem?

The probability that in a litter of six kittens, three will be black and three will be gray.

What information is provided to solve the problem?

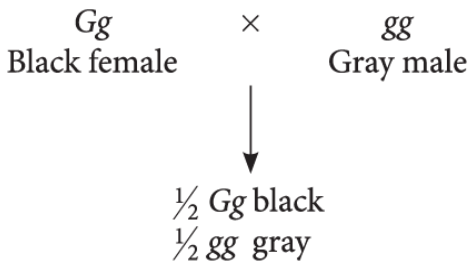
- Black is dominant to gray.
- The mother of the litter is black and her mother is gray.
- The father of the litter is gray.

For help with this problem, review:

The Binomial Expansion and Probability in **Section 3.2**.

» Solution Steps

Because black (*G*) is dominant to gray (*g*), a black cat may be homozygous (*GG*) or heterozygous (*Gg*). The black female in this problem must be heterozygous (*Gg*) because her mother is gray (*gg*) and she must have inherited one of her mother's alleles. The gray male must be homozygous (*gg*) because gray is recessive. Thus, the cross is



Description
The P generation shows a black female with genotype big G small G is crossed with a gray male with genotype small G small G. The genotypes of the progenies are as follows: one half big G small G black and one half small G small G gray.

Hint: We can determine the female parent's genotype from her phenotype and her mother's phenotype.

We can use the binomial expansion to determine the probability of obtaining three black and three gray kittens in a litter of six. Let *p* equal the probability of a kitten being black and *q* equal the probability of a kitten being gray. The binomial is $(p + q)^6$, the expansion of which is

Recall: The binomial expansion can be used to determine the probability of different combinations of traits in the progeny of a cross.

$$(p+q)^6 = p^6 + 6p^5q^1 + 15p^4q^2 + 20p^3q^3 + 15p^2q^4 + 6p^1q^5 + q^6$$

Hint: See the discussion of Binomial Expansion and Probability for an explanation of how to expand the binomial.

The probability of obtaining three black and three gray kittens in a litter of six is provided by the term $20p^3q^3$. The probabilities of p and q are both $^1/_2$, so the overall probability is $20(^1/_2)^3(^1/_2)^3 = {}^{20}/_{64} = {}^5/_{16}$.

Problem 3

In corn, purple kernels are dominant to yellow kernels, and full kernels are dominant to shrunken kernels. A corn plant that has purple and full kernels is crossed with a plant that has yellow and shrunken kernels, and the following progeny are obtained:

purple, full	112
purple, shrunken	103
yellow, full	91
yellow, shrunken	94

What are the most likely genotypes of the parents and progeny? Test your genetic hypothesis with a chi-square test.

» Solution Strategy

What information is required in your answer to the problem?

a. The genotypes of parents and progeny.
b. A chi-square test comparing the observed and expected results and the interpretation of the chi-square test.

What information is provided to solve the problem?

- Purple kernels are dominant to yellow kernels, and full kernels are dominant to shrunken kernels.
- The phenotypes of the parents.
- The phenotypes and numbers of progeny of the cross.

For help with this problem, review:

Sections 3.3 and 3.4.

» Solution Steps

The best way to begin this problem is by breaking the cross down into simple crosses for a single characteristic (kernel color or kernel shape):

P	purple × yellow	full × shrunken
F_1	112 + 103 = 215 purple	112 + 91 = 203 full
	91 + 94 = 185 yellow	103 + 94 = 197 shrunken

Hint: A good strategy in a cross involving multiple characteristics is to analyze the results for each characteristic separately.

In this cross, purple × yellow produces approximately $^1/_2$ purple and $^1/_2$ yellow (a 1:1 ratio). A 1:1 ratio is usually the result of a cross between a heterozygote and a homozygote. Because purple is dominant, the purple parent must be heterozygous (*Pp*) and the yellow parent must be homozygous (*pp*). The purple progeny produced by this cross will be heterozygous (*Pp*), and the yellow progeny must be homozygous (*pp*).

Now let's examine the other character. Full × shrunken produces $^1/_2$ full and $^1/_2$ shrunken, or a 1:1 ratio, so these progeny phenotypes are also produced by a cross between a heterozygote (*Ff*) and a homozygote (*ff*); the full-kernel progeny will be heterozygous (*Ff*), and the shrunken-kernel progeny will be homozygous (*f f*).

Now combine the two crosses and use the multiplication rule to obtain the overall genotypes and the proportions of each genotype:

Recall: The multiplication rule states that the probability of two or more independent events occurring together is calculated by multiplying their independent probabilities.

P	Purple, full *Pp Ff*	×	Yellow, shrunken *pp ff*
F_1	*Pp Ff* = ½ purple	×	½ full = ¼ purple, full
	Pp ff = ½ purple	×	½ shrunken = ¼ purple, shrunken
	Pp Ff = ½ yellow	×	½ full = ¼ yellow, full
	Pp ff = ½ yellow	×	½ shrunken = ¼ yellow, shrunken

Description
The P generation at the top shows a purple full parent with genotype big P small P big F small F is crossed with a yellow shrunken parent with genotype small P small P small F small F. The F subscript 1 generation obtained below is as follows:

Big P small P big F small F equals one half purple crossed with one half full equals one fourth purple, full.

Big P small P small F small F equals one half purple crossed with one half shrunken equals one fourth purple, shrunken.

Big P small P big F small F equals one half yellow crossed with one half full equals one fourth yellow, full.

Big P small P small F small F equals one half yellow crossed with one half shrunken equals one fourth yellow, shrunken.

Our genetic calculations predict that from this cross, we should see $^1/_4$ purple, full-kernel progeny; $^1/_4$ purple, shrunken-kernel progeny; $^1/_4$ yellow, full-kernel progeny; and $^1/_4$ yellow, shrunken-kernel progeny. A total of 400 progeny were produced, so $^1/_4 \times 400 = 100$ of

each phenotype are expected. Therefore, the observed numbers do not fit the expected numbers exactly.

Could the difference between what we observe and what we expected be due to chance? If the probability is high that chance alone is responsible for the difference between observed and expected values, we will assume that the progeny have been produced in the 1:1:1:1 ratio predicted for the cross. If the probability is low that the difference between observed and expected values is due to chance, we will assume that the progeny really are not in the predicted ratio and that some other, *significant* factor must be responsible for the deviation from our expectations.

The observed and expected numbers are

Phenotype	Observed	Expected
purple, full	112	$^1/_4 \times 400 = 100$
purple, shrunken	103	$^1/_4 \times 400 = 100$
yellow, full	91	$^1/_4 \times 400 = 100$
yellow, shrunken	94	$^1/_4 \times 400 = 100$

To determine the probability that the difference between observed and expected numbers is due to chance, we calculate a chi-square

value using the formula

$$\begin{aligned}
\chi^2 &= \Sigma[(\text{observed} - \text{expected})^2/\text{expected}]: \\
\chi^2 &= \frac{(112-100)^2}{100} + \frac{(103-100)^2}{100} \\
&\quad + \frac{(91-100)^2}{100} + \frac{(94-100)^2}{100} \\
&= \frac{12^2}{100} + \frac{3^2}{100} + \frac{9^2}{100} + \frac{6^2}{100} \\
&= \frac{144}{100} + \frac{9}{100} + \frac{81}{100} + \frac{36}{100} \\
&= 1.44 + 0.09 + 0.81 + 0.36 = 2.70
\end{aligned}$$

Hint: See Figure 3.15 for help on how to carry out a chi-square test.

The next step is to determine the probability associated with this calculated chi-square value, which is the probability that the deviation between the observed and the expected results is due to chance. This step requires us to compare our calculated chi-square value (2.70) with theoretical values in a chi-square table that have the same degrees of freedom. The degrees of freedom for a chi-square goodness-of-fit test are $n - 1$, where n equals the number of expected phenotypic classes. In this case, there are four expected phenotypic classes, so the degrees of freedom equal 4 − 1 = 3. We must now look up the chi-square value in a chi-square table (see Table 3.7). We select the row corresponding to 3 degrees of freedom and look along this row to find our calculated chi-square value. The

calculated chi-square value of 2.7 lies between 2.366 (a probability of 0.5) and 6.251 (a probability of 0.1). The probability (*P*) associated with the calculated chi-square value is therefore $0.5 > P > 0.1$. This *P* is the probability that the difference between what we observed and what we expected is due to chance, which in this case is relatively high, so chance is probably responsible for the deviation. We can conclude that the progeny *do* appear in the 1:1:1:1 ratio predicted by our genetic explanation.

IMPORTANT TERMS

gene
allele
locus
genotype
homozygous
heterozygous
phenotype
monohybrid cross
P (parental) generation
F_1 (first filial) generation
reciprocal cross
F_2 (second filial) generation
dominant
recessive
principle of segregation (Mendel's first law)
concept of dominance

chromosome theory of heredity
backcross
Punnett square
probability
multiplication rule
addition rule
conditional probability
testcross
wild type
dihybrid cross
principle of independent assortment (Mendel's second law)
chi-square goodness-of-fit test

ANSWERS TO CONCEPT CHECKS

1. b
2. A locus is a place on a chromosome where genetic information encoding a characteristic is located. An allele is a version of a gene that encodes a specific trait. A genotype is the set of alleles possessed by an individual organism, and a phenotype is the manifestation or appearance of a characteristic.
3. The traits encoded by both alleles appeared in the F_2 progeny.
4. d
5. a
6. Both the principle of segregation and the principle of independent assortment refer to the separation of alleles in anaphase I of meiosis. The principle of segregation says that

these alleles separate, and the principle of independent assortment says that they separate independently of alleles at other loci.

7. d

Chapter 3 Assessment

COMPREHENSION QUESTIONS

Section 3.1

1. Why was Mendel's approach to the study of heredity so successful?

2. What is the difference between genotype and phenotype?

Section 3.2

3. What is the principle of segregation? Why is it important?

4. CONNECT How are Mendel's principles different from the concept of blending inheritance discussed in Chapter 1?

5. What is the concept of dominance?

6. What are the addition and multiplication rules of probability, and when should they be used?

7. Give the genotypic ratios that may appear among the progeny of simple crosses and the genotypes of the parents that may give rise to each ratio.

8. What is the chromosome theory of heredity? Why was it important?

Section 3.3

9. What is the principle of independent assortment? How is it related to the principle of segregation?

10.

In which stages of mitosis and meiosis are the principles of segregation and independent assortment at work?

Section 3.4

11. How is the chi-square goodness-of-fit test used to analyze genetic crosses? What does the probability associated with a chi-square value indicate about the results of a cross?

APPLICATION QUESTIONS AND PROBLEMS

Introduction

12. If blond hair in the Solomon Islanders had originated from early European explorers, what would you predict the researchers

would have found when they conducted their genetic study of the islanders?

Section 3.1

13. *What characteristics of an organism would make it suitable for studies of the principles of inheritance? Name several organisms that have these characteristics.

Section 3.2

14. *In cucumbers, orange fruit color (*R*) is dominant to cream fruit color (*r*). A cucumber plant homozygous for orange fruit is crossed with a plant homozygous for cream fruit. The F_1 are intercrossed to produce the F_2.

    a. Give the genotypes and phenotypes of the parents, the F_1, and the F_2.

    b. Give the genotypes and phenotypes of the offspring of a backcross between the F_1 and the orange-fruited parent.

    c. Give the genotypes and phenotypes of a backcross between the F_1 and the cream-fruited parent.

15. CONNECT **Figure 1.1** shows three girls, one of whom has albinism. Could the three girls shown in the photograph be sisters? Why or why not?

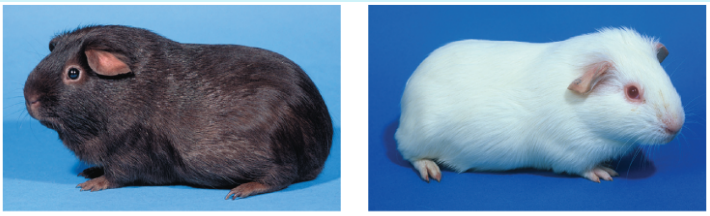
16. DATA ANALYSIS J. W. McKay crossed a stock melon plant that produced tan seeds with a plant that produced red seeds and obtained the following results (J. W. McKay. 1936. *Journal of Heredity* 27:110–112).

Cross	F_1	F_2
tan ♀ × red ♂	13 tan seeds	93 tan, 24 red, seeds

a. Explain the inheritance of tan and red seeds in this plant.

b. Assign symbols for the alleles in this cross, and give genotypes for all the individual plants.

17. DATA ANALYSIS CONNECT *White (*w*) coat color in guinea pigs is recessive to black (*W*). In 1909, W. E. Castle and J. C. Phillips transplanted an ovary from a black guinea pig into a white female whose ovaries had been removed. They then mated this white female with a white male. All the offspring from the mating were black (W. E. Castle and J. C. Phillips. 1909. *Science* 30:312–313).



Wegner/ARCO/Nature Picture Library; Nigel Cattlin/Alamy.

a. Explain the results of this cross.

b. Give the genotype of the offspring of this cross.

c. What, if anything, does this experiment indicate about the validity of the pangenesis and the germ-plasm theories discussed in Chapter 1?

18. *In cats, blood type A results from an allele (I^A) that is dominant to an allele (i^B) that produces blood type B. There is no O blood type. The blood types of male and female cats that were mated and the blood types of their kittens follow. Give the most likely genotypes for the parents of each litter.

	Male parent	Female parent	Kittens
a.	A	B	4 with type A, 3 with type B
b.	B	B	6 with type B
c.	B	A	8 with type A
d.	A	A	7 with type A, 2

			with type B
e.	A	A	10 with type A
f.	A	B	4 with type A, 1 with type B

19. Figure 3.8 shows the results of a cross between a tall pea plant and a short pea plant.

    a. What phenotypes and proportions will be produced if a tall F_1 plant is backcrossed to the short parent?

    b. What phenotypes and proportions will be produced if a tall F_1 plant is backcrossed to the tall parent?

20. Joe has a white cat named Sam. When Joe crosses Sam with a black cat, he obtains $^1/_2$ white kittens and $^1/_2$ black kittens. When the black kittens are interbred, all the kittens that they produce are black. On the basis of these results, would you conclude that white or black coat color in cats is a recessive trait? Explain your reasoning.

21. *In sheep, lustrous fleece results from an allele (L) that is dominant to an allele (l) for normal fleece. A ewe (adult female) with lustrous fleece is mated with a ram (adult male) with

normal fleece. The ewe then gives birth to a single lamb with normal fleece. From this single offspring, is it possible to determine the genotypes of the two parents? If so, what are their genotypes? If not, why not?

Jeffrey van Daele/FeaturePics.

22. *Alkaptonuria is a metabolic disorder in which affected people produce black urine. Alkaptonuria results from an allele (*a*) that is recessive to the allele for normal metabolism (*A*). Sally has normal metabolism, but her brother has alkaptonuria. Sally's

father has alkaptonuria, and her mother has normal metabolism.

a. Give the genotypes of Sally, her mother, her father, and her brother.

b. If Sally's parents have another child, what is the probability that this child will have alkaptonuria?

c. If Sally marries a man with alkaptonuria, what is the probability that their first child will have alkaptonuria?

23. In rabbits, coat color is a genetically determined trait. Some black females always produce black progeny, whereas other black females produce black progeny and white progeny. Explain how this occurs.

24. Hairlessness in American rat terriers is recessive to the presence of hair. Suppose that you have a rat terrier with hair. How can you determine whether this dog is homozygous or heterozygous for the hairy trait?

25. *What is the probability of rolling one six-sided die and obtaining the following numbers?

a. 2

b. 1 or 2

c. An even number

d. Any number but a 6

26. *What is the probability of rolling two six-sided dice and obtaining the following numbers?

    a. 2 and 3

    b. 6 and 6

    c. At least one 6

    d. Two of the same number (two 1s, two 2s, two 3s, etc.)

    e. An even number on both dice

    f. An even number on at least one die

27. *In a family of seven children, what is the probability of obtaining the following distribution of boys and girls?

    a. All boys

    b. All children of the same sex

    c. Six girls and one boy

    d. Four boys and three girls

    e. Four girls and three boys

28. Phenylketonuria (PKU) is a disease that results from a recessive gene. Suppose that two unaffected parents produce a child with PKU.

a. What is the probability that a sperm from the father will contain the PKU allele?

b. What is the probability that an egg from the mother will contain the PKU allele?

c. What is the probability that their next child will have PKU?

d. What is the probability that their next child will be heterozygous for the PKU gene?

29. CONNECT *In German cockroaches, curved wings (*cv*) are recessive to normal wings (cv^+). A homozygous cockroach having normal wings is crossed with a homozygous cockroach having curved wings. The F_1 are intercrossed to produce the F_2. Assume that the pair of chromosomes containing the locus for wing shape is metacentric. Draw this pair of chromosomes as it would appear in the parents, the F_1, and each class of F_2 progeny at metaphase I of meiosis. Assume that no crossing over takes place. At each stage, label a location for the alleles for wing shape (*cv* and cv^+) on the chromosomes.

30. CONNECT *In guinea pigs, the allele for black fur (*B*) is dominant to the allele for brown (*b*) fur. A black guinea pig is crossed with a brown guinea pig, producing five F_1 black guinea pigs and six F_1 brown guinea pigs.

a. How many copies of the black allele (*B*) will be present in each cell of an F_1 black guinea pig at the following stages: G_1, G_2, metaphase of mitosis, metaphase I of meiosis, metaphase II of meiosis, and after the second cytokinesis following meiosis? Assume that no crossing over takes place.

b. How many copies of the brown allele (*b*) will be present in each cell of an F_1 brown guinea pig at the same stages as those listed in part *a*? Assume that no crossing over takes place.

Section 3.3

31. In watermelons, bitter fruit (*B*) is dominant to sweet fruit (*b*), and yellow spots (*S*) are dominant to no spots (*s*). The genes for these two characteristics assort independently. A homozygous plant that has bitter fruit and yellow spots is crossed with a homozygous plant that has sweet fruit and no spots. The F_1 are intercrossed to produce the F_2.

    a. What are the phenotypic ratios in the F_2?

    b. If an F_1 plant is backcrossed with the bitter, yellow-spotted parent, what phenotypes and proportions are expected in the offspring?

    c. If an F_1 plant is backcrossed with the sweet, unspotted parent, what phenotypes and proportions are expected in the

offspring?

32. **Figure 3.11** shows the results of a dihybrid cross involving seed shape and seed color.

    a. What proportion of the round and yellow F_2 progeny from this cross is homozygous at both loci?

    b. What proportion of the round and yellow F_2 progeny from this cross is homozygous at least at one locus?

33. *In cats, curled ears result from an allele (*Cu*) that is dominant to an allele (*cu*) for normal ears. Black color results from an independently assorting allele (*G*) that is dominant to an allele for gray (*g*). A gray cat homozygous for curled ears is mated with a homozygous black cat with normal ears. All the F_1 cats are black and have curled ears.

    a. If two of the F_1 cats mate, what phenotypes and proportions are expected in the F_2?

    b. An F_1 cat mates with a stray cat that is gray and possesses normal ears. What phenotypes and proportions of progeny are expected from this cross?

Jean-Michel Labat/Science Source.

34. *The following two genotypes are crossed: *Aa Bb Cc dd Ee* × *Aa bb Cc Dd Ee*. What will the proportion of the following genotypes be among the progeny of this cross?

    a. *Aa Bb Cc Dd Ee*

    b. *Aa bb Cc dd ee*

    c. *aa bb cc dd ee*

    d. *AA BB CC DD EE*

35. In mice, an allele for apricot eyes (*a*) is recessive to an allele for brown eyes (a^+). At an independently assorting locus, an allele

for tan coat color (t) is recessive to an allele for black coat color (t^+). A mouse that is homozygous for brown eyes and black coat color is crossed with a mouse having apricot eyes and a tan coat. The resulting F_1 are intercrossed to produce the F_2. In a litter of eight F_2 mice, what is the probability that two will have apricot eyes and tan coats?

36. In cucumbers, dull fruit (D) is dominant to glossy fruit (d), orange fruit (R) is dominant to cream fruit (r), and bitter cotyledons (B) are dominant to non-bitter cotyledons (b). The three characters are encoded by genes located on different pairs of chromosomes. A plant homozygous for dull, orange fruit and bitter cotyledons is crossed with a plant that has glossy, cream fruit and non-bitter cotyledons. The F_1 are intercrossed to produce the F_2.

    a. Give the phenotypes and their expected proportions in the F_2.

    b. An F_1 plant is crossed with a plant that has glossy, cream fruit and non-bitter cotyledons. Give the phenotypes and expected proportions among the progeny of this cross.

37.

*Alleles *A* and *a* are located on a pair of metacentric chromosomes. Alleles *B* and *b* are located on a pair of acrocentric chromosomes (see Chapter 2). A cross is made between individuals having the following genotypes: *Aa Bb* × *aa bb*.

a. Draw the chromosomes as they would appear in each type of gamete produced by these individuals.

b. For each type of progeny resulting from this cross, draw the chromosomes as they would appear in a cell at G_1, G_2, and metaphase of mitosis.

Section 3.4

38. *J. A. Moore investigated the inheritance of spotting patterns in leopard frogs (J. A. Moore. 1943. *Journal of Heredity* 34:3–7). The pipiens phenotype had the normal spots that give leopard frogs their name. In contrast, the burnsi phenotype lacked spots on its back. Moore carried out the following crosses, producing the progeny indicated.

Parent phenotypes	Progeny phenotypes
burnsi × burnsi	39 burnsi, 6 pipiens
burnsi × pipiens	23 burnsi, 33 pipiens
burnsi × pipiens	196 burnsi, 210 pipiens

a. On the basis of these results, is the burnsi phenotype most likely inherited as a dominant trait or as a recessive trait?

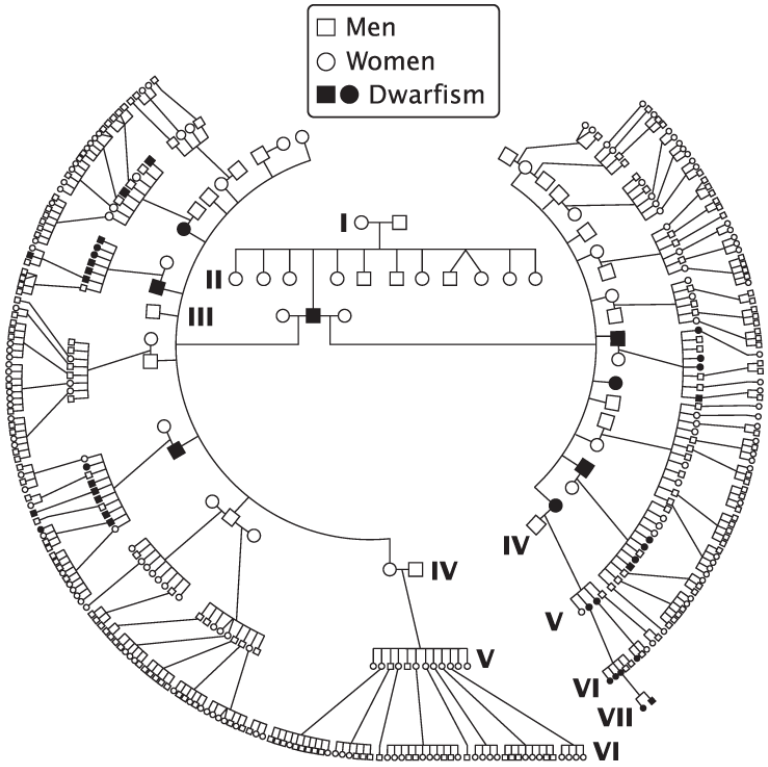
b. Give the most likely genotypes of the parent in each cross (use *B* for the burnsi allele and B^+ for the pipiens allele).

c. Use a chi-square test to evaluate the fit of the observed numbers of progeny to the number expected on the basis of your proposed genotypes.

39. In the 1800s, a man with dwarfism who lived in Utah produced a large number of descendants: 22 children, 49 grandchildren, and 250 great-grandchildren (see the illustration of a family pedigree below), many of whom also exhibited dwarfism (F. F. Stephens. 1943. *Journal of Heredity* 34:229–235). The type of dwarfism found in this family is called Schmid-type metaphyseal chondrodysplasia, although it was originally thought to be achondroplastic dwarfism. Among the families of this kindred, dwarfism appeared only in members who had one parent with dwarfism. When one parent exhibited dwarfism, the following numbers of children were produced.

Family in which one parent had dwarfism	Children with normal stature	Children with dwarfism
A	15	7
B	4	6
C	1	6

D	6	2
E	2	2
F	8	4
G	4	4
H	2	1
I	0	1
J	3	1
K	2	3
L	2	1
M	2	0
N	1	0
O	0	2
Total	52	40



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[Data from *the Journal of Heredity* 34:232.]

Description
Generations 1 through 3 are in the center and generations 4 through 6 have large number of people. They are drawn as circles around the first 3 generations. Men are represented as squares and women as circles. The trait for dwarfism is shown with a filled circle or square. Generation 3 has one male dwarf. The male in generation 3 has 4 dwarf children out of 11 with one female and 3 dwarf children out of 10 with another female. None of the non-dwarfs had dwarf children. The people who were dwarfs had some children who were dwarfs.

a. With the assumption that Schmid-type metaphyseal chondrodysplasia is rare, is this type of dwarfism inherited as a dominant or a recessive trait? Explain your reasoning.

b. On the basis of your answer for part *a*, what is the expected ratio of children with dwarfism to children with normal stature in the families given in the table? Use a chi-square test to determine if the total number of children with each phenotype in these families (52 with normal stature, 40 with dwarfism) is significantly different from the number expected.

c. Use chi-square tests to determine if the numbers of children with each phenotype in family C (1 with normal stature, 6 with dwarfism) and in family D (6 with normal stature, 2 with dwarfism) are significantly different from the numbers expected on the basis of your proposed mode of inheritance. How would you explain these deviations from the overall ratio expected?

40.

Pink-eye and albino are two recessive traits found in the deer mouse *Peromyscus maniculatus*. In pink-eyed mice, the eye is devoid of color and appears pink because of the blood vessels within it. Albino mice are completely lacking color, both in their fur and in their eyes. F. H. Clark crossed pink-eyed mice with albino mice; the resulting F_1 had normal coloration in their fur and eyes. He then crossed these F_1 mice with mice that were pink eyed and albino and obtained the following progeny. It is hard to distinguish between mice that are albino and mice that are both pink eyed and albino, so he combined these two phenotypes (F. H. Clark. 1936. *Journal of Heredity* 27:259–260).

Phenotype	**Number of progeny**

wild-type fur, wild-type eye color	12
wild-type fur, pink-eye	62
albino albino, pink-eye }	78
Total	152

a. Give the expected numbers of progeny with each phenotype if the genes for pink-eye and albino assort independently.

b. Use a chi-square test to determine if the observed numbers of progeny fit the number expected with independent assortment.

41. *In the California poppy, an allele for yellow flowers (*C*) is dominant to an allele for white flowers (*c*). At an independently assorting locus, an allele for entire petals (*F*) is dominant to an allele for fringed petals (*f*). A plant that is homozygous for yellow and entire petals is crossed with a plant that has white and fringed petals. A resulting F_1 plant is then crossed with a plant that has white and fringed petals, and the following progeny are produced: 54 yellow and entire; 58 yellow and fringed; 53 white and entire; 10 white and fringed.

a. Use a chi-square test to compare the observed numbers of progeny having each phenotype with those expected for the cross.

b. What conclusion can you draw from the results of the chi-square test?

c. Suggest an explanation for the results.

CHALLENGE QUESTIONS

Section 3.2

42. Dwarfism is a recessive trait in Hereford cattle; the gene causing this trait has not been identified. A rancher in western Texas discovers that several of the calves in his herd are dwarfs, and he wants to eliminate this undesirable trait from the herd as rapidly as possible. Suppose that the rancher hires you as a genetic consultant to advise him on how to breed the dwarfism trait out of the herd. What crosses would you advise the rancher to conduct to ensure that the allele causing dwarfism is eliminated from the herd?

43. *A geneticist discovers an obese mouse in his laboratory colony. He breeds this obese mouse with a normal mouse. All the F_1 mice from this cross are normal in size. When he interbreeds two F_1 mice, eight of the F_2 mice are normal in size and two are obese. The geneticist then intercrosses two of his obese mice, and he finds that all the progeny from this cross are obese. These results lead the geneticist to conclude that obesity in mice results from a recessive allele.

A second geneticist at a different university also discovers an obese mouse in her laboratory colony. She carries out the same crosses as the first geneticist and obtains the same results. She also concludes that obesity in mice results from a recessive allele.

One day the two geneticists meet at a genetics conference, learn of each other's experiments, and decide to exchange mice. They both find that when they cross two obese mice from the different laboratories, all the offspring are normal; however, when they cross two obese mice from the same laboratory, all the offspring are obese. Explain their results.

44.

Albinism is a recessive trait in humans (see the introduction to Chapter 1). A geneticist studies a series of families in which both parents have pigmentation and at least one child has albinism. The geneticist reasons that both parents in these families must be heterozygotes and that albinism should appear in $^1/_4$ of their children. To his surprise, the geneticist finds that the frequency of albinism among the children of these families is significantly greater than $^1/_4$. Can you think of an explanation for the higher-than-expected frequency of albinism among these families?

45. Two distinct phenotypes are found in the salamander *Plethodon cinereus*: a red form and a black form. Some biologists have speculated that the red phenotype is due to an allele that is dominant to an allele for black. Unfortunately, these

salamanders will not mate in captivity, so the hypothesis that red is dominant to black has never been tested.

One day, a genetics student is hiking through the forest and finds 30 female salamanders, some red and some black, laying eggs. The student places each female with her eggs (about 20 to 30 eggs per female) in a separate plastic bag and takes them back to the lab. There, the student successfully raises the eggs until they hatch. After the eggs have hatched, the student records the phenotypes of the juvenile salamanders, along with the phenotypes of their mothers. Thus, the student has the phenotypes for 30 females and their progeny, but no information is available about the phenotypes of the fathers.

Explain how the student can determine whether red is dominant to black with this information on the phenotypes of the females and their offspring.

George Grall/Getty Images.

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Introduction

1. About 40% of Solomon Islanders carry a gene for blond hair, and yet only 5%–10% of these people actually have blond hair. Why is the proportion of people with blond hair only 5%–10% when so many people carry genes for blond hair?

Section 3.1

2. Why was Mendel's success dependent on his studying characteristics that exhibit only two easily distinguished phenotypes, such as white versus gray seed coats and round versus wrinkled seeds? Would he have been less successful if he had instead studied traits such as seed weight or leaf length, which vary much more in their phenotypes? Explain your answer.

Section 3.2

3. Geneticists often carry out reciprocal crosses when they are studying the inheritance of traits. Why do geneticists use reciprocal crosses?

4. Red hair in humans is inherited as a recessive trait. Bill and Sarah both have black hair. They marry and have four children, three of whom have red hair. Bill says it isn't genetically possible for two black-haired people to have $^3/_4$ red-haired children, and he accuses Sarah of infidelity. Sarah asserts that Bill doesn't understand genetics and probability, and that it is possible for two black-haired people to have $^3/_4$ red-haired children. Who is correct and why?

5. Suppose that you are raising Mongolian gerbils. You notice that some of your gerbils have white spots, whereas others have solid coats. What type of crosses could you carry out to determine whether white spots are due to a recessive or a dominant allele?

Section 3.3

6. Are Mendel's principles of segregation and independent assortment even relevant today in the age of genomics, when it is possible to sequence an organism's entire genome and determine all of its genetic information? Why is it important to study these principles, and how can they be used?

7. In cats, short hair (*L*) is dominant to long hair (*l*), and stripes (*A*) are dominant to solid color (*a*). A cat with genotype *Ll Aa* mates with a cat that is *Ll aa*, and they produce a litter of six kittens. What is the probability that four of the six kittens will have both long hair and stripes?

Section 3.4

8. In corn, purple kernels (*P*) are dominant to yellow kernels (*p*), and starchy kernels (*Su*) are dominant to sugary kernels (*su*). A corn plant grown from a purple and starchy kernel is crossed with a plant grown from a yellow and sugary kernel, and the following progeny (kernels) are produced:

Phenotype	Number
purple, starchy	150
purple, sugary	142
yellow, starchy	161

yellow, sugary	115

Formulate a hypothesis about the genotypes of the parents and offspring in this cross. Perform a chi-square goodness-of-fit test comparing the observed numbers of progeny with the numbers expected based on your genetic hypothesis. What conclusion can you draw based on the results of your chi-square test? Can you suggest an explanation for the observed results?

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Draw a concept map that explains the genetic basis of blond hair in Solomon Islanders using the following terms: Solomon Islanders, Europeans, blond hair, recessive trait, dark hair, melanin, chromosome 9, dominant trait, *TYRP1*, *KITLG*, *TT*, *CT*, *CC*.

Section 3.1

2. Outline the relationships among important genetic terminology by creating a concept map that relates the following terms: phenotype, gene, homozygous, heterozygous, locus, allele, genotype.

Section 3.2

3. Using a concept map, compare and contrast the principle of segregation (Mendel's first law) and the principle of independent assortment (Mendel's second law).

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 4

Sex Determination and Sex-Linked Characteristics

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In European traditions, dragons are often portrayed as large lizards. This statue adorns the Dragon Bridge in Ljubljana, Slovenia.

The Sex of a Dragon

Dragons are the stuff of kids' tales and nightmares—giant fire-breathing reptiles that destroy everything in their path. These mythical creatures have a long history, appearing in folk traditions of

both Europe and Asia. Almost always reptilian, dragons were originally depicted as snakelike, but since the Middle Ages they have been increasingly drawn with legs, often imagined as large lizards. In European traditions, dragons are usually evil, but in Asian myths, dragons are a symbol of benevolent power and sometimes of fertility.

Real dragons are found in the deserts of central Australia. Known as central bearded dragons (*Pogona vitticeps*), these lizards grow up to two feet in length (**Figure 4.1**). Like some of their mythical relatives, they possess numerous spines that run from head to tail. Their color varies widely, from brown to gray, red, yellow, orange, or even white. Bearded dragons derive their name from a pouch that projects from the underside of the chin, often appearing dark in color,like a man's beard. When threatened, they open their mouths and hiss, imitating their mythological namesake (but, disappointingly, without the fire). But it is through their sex that bearded dragons have become best known to geneticists.

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4.1 A central bearded dragon (*Pogona vitticeps*).

As we learn in this chapter, organisms employ an amazing variety of mechanisms to determine sex—whether an organism is male or female. In some, such as humans, sex is determined by chromosomes: females possess two identical sex chromosomes (X chromosomes), and males possess a single X and a different chromosome, called Y. In others, such as birds, butterflies, and some reptiles, sex is still chromosomal, but it is the male that possesses two identical sex chromosomes (called Z chromosomes) and the female that has two different sex chromosomes (called Z and W). There are even some animals for which sex is not determined by sex chromosomes at all but is primarily a function of the environment: in turtles and alligators, sex is influenced by the temperature at which the eggs are incubated, with males produced at one temperature and females at another.

For many years, scientists thought that sex determination could be genetic or environmental, but not both. In 2015, however, scientists reported the results of field and laboratory studies demonstrating unequivocally that sex in bearded dragons is determined by sex chromosomes *and* the temperature at which their eggs are incubated.

A female bearded dragon lays a clutch of 11 to 30 eggs in a nest in the sand. The female then leaves the nest, and the eggs develop unattended, warmed by the desert sun. Eventually, baby dragons emerge from the eggs.

Bearded dragons have sex chromosomes: males are normally ZZ and females are ZW. Scientists discovered, however, that over 20% of wild-caught females examined in one year had ZZ sex chromosomes, which are expected only in males. The scientists found that when they incubated eggs with ZZ embryos at temperatures below 32°C, the offspring were all males (as expected), but as they increased the incubation temperature, more and more ZZ embryos developed as females. At 36°C, almost all the ZZ individuals were females. They concluded that ZZ females found in the wild probably hatched from warm nests. Matings between ZZ males and ZZ females produced viable and fertile offspring, all ZZ; for unknown reasons, ZZ females themselves lay twice as many eggs as normal ZW females. In contrast to ZZ lizards, which can be male or female depending on incubation temperature, ZW lizards are always female. One concern of the researchers was how these lizards might respond to future

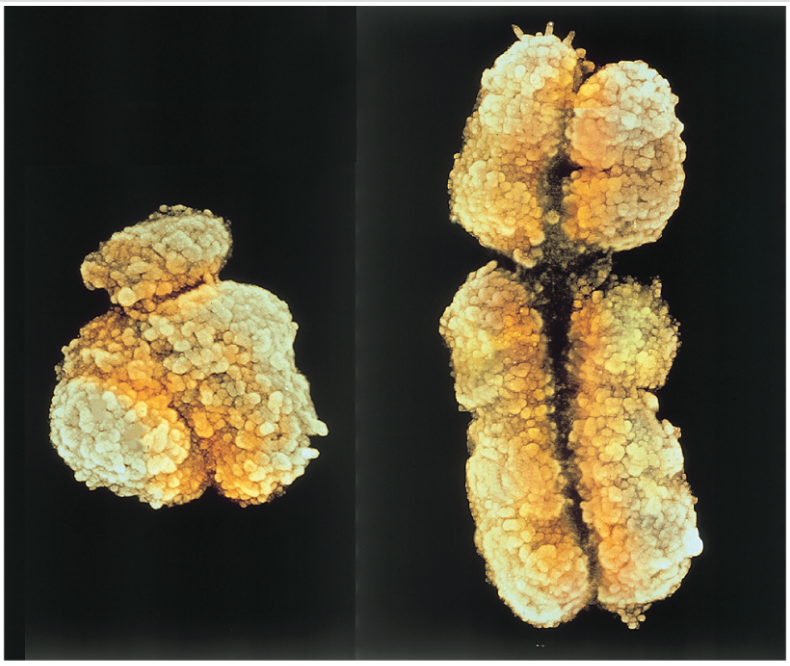
climate change. If the environment warms significantly, only females may be produced, leading to the extinction of the species.

THINK-PAIR-SHARE

- Climate warming could lead to all-female populations of bearded dragons, resulting in extinction of the species. What are some other potential effects of climate warming on natural populations of organisms?
- High incubation temperatures reverse the sex of ZZ bearded dragons, causing them to develop as females instead of males. Imagine that temperature also caused sex reversal in ZW individuals, so that high temperatures caused ZW individuals to develop as males instead of females (this doesn't actually happen in bearded dragons). What would be the result of a mating between a normal ZW female and a sex-reversed ZW male?

In Chapter 3, we studied Mendel's principles of segregation and independent assortment and saw how these concepts explain much about the nature of inheritance. After Mendel's principles were rediscovered in 1900, biologists began to conduct genetic studies on a wide array of different organisms. As they applied Mendel's principles more widely, they observed exceptions that made it necessary to devise extensions of those basic principles. In this chapter, we explore one of those extensions: the inheritance of characteristics encoded by genes located on the sex chromosomes, which often differ between males and females (**Figure 4.2**). These characteristics, and the genes that produce them, are referred to as sex linked. To understand the inheritance of sex-linked characteristics, we must first know how sex is determined—why some members of a species are male and others are female. Thus, the first part of this chapter focuses on sex determination. The

second part examines how characteristics encoded by genes on the sex chromosomes are inherited and some of the consequences of this inheritance. In Chapter 5, we will explore some additional ways in which sex and inheritance interact.



Biophoto Associates/Science Source.

4.2 The human male sex chromosome (Y, at the left) differs from the human female sex chromosome (X, at the right) in size and shape.

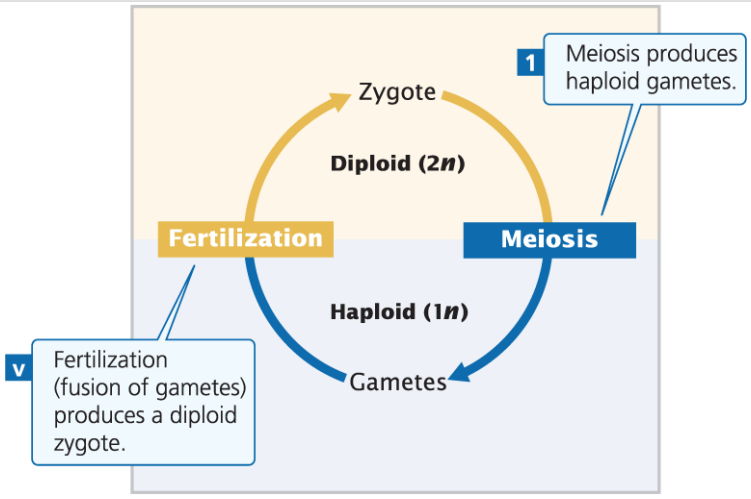
Description
The Y-chromosome has 3 distinct thick sections clumped together and is relatively half the size of the elongated X-chromosome with 4 sections.

As we consider sex determination and sex-linked characteristics, it will be helpful to keep two important principles in mind. First, there are several different mechanisms of sex determination, and ultimately, a species' mechanism of sex determination controls its

inheritance of sex-linked characteristics. Second, like other pairs of chromosomes, the X and Y sex chromosomes pair in the course of meiosis and segregate, but throughout most of their length, they are not homologous (their gene sequences do not encode the same characteristics): most genes on the X chromosome are different from genes on the Y chromosome. Consequently, males and females do not possess the same numbers of alleles at sex-linked loci. This difference in the number of sex-linked alleles produces distinct patterns of inheritance in males and females.

4.1 Sex Is Determined by a Number of Different Mechanisms

Sexual reproduction is the formation of offspring that are genetically distinct from their parents; most often, two parents contribute genes to their offspring, and those genes are assorted into new combinations through meiosis. Among most eukaryotes, sexual reproduction consists of two processes that lead to an alternation of haploid and diploid cells: meiosis produces haploid gametes (spores in plants), and fertilization produces diploid zygotes (Figure 4.3).



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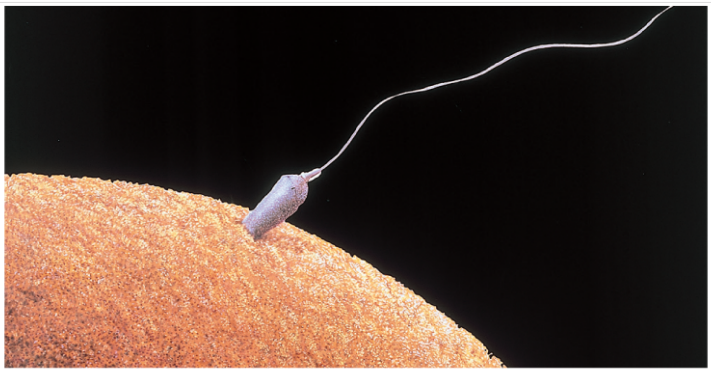
4.3 In most eukaryotic organisms, sexual reproduction consists of an alternation of haploid (1*n*) and diploid (2*n*) cells.

Description

The illustration is divided into two parts diploid (2 n) and haploid (1 n). It starts with zygote in diploid stage, which undergoes meiosis to produce gametes that are haploid. A caption

reads, Meiosis produces haploid gametes. Gametes then undergo fertilization to produce zygote. A caption reads, Fertilization (fusion of gametes) produces a diploid zygote. The cycle repeats.

The term **sex** refers to sexual phenotype. Most organisms have two sexual phenotypes: male and female. The fundamental difference between males and females is gamete size: males produce small gametes, and females produce relatively large gametes (**Figure 4.4**).



Francis Leroy, Biocosmos/Science Source.

4.4 Male and female gametes differ in size. In this photograph, a human sperm (with flagellum) penetrates a human egg.

The mechanism by which biological sex is established is termed **sex determination**. We define the sex of an individual organism in reference to its phenotype. Sometimes an individual organism has chromosomes or genes that are normally associated with one sex but an anatomy corresponding to the opposite sex. For instance, the cells of human females normally have two X chromosomes, and the cells of males have one X chromosome and one Y chromosome. A few rare individuals have male anatomy although their cells each

contain two X chromosomes. Even though these people are genetically female, we refer to them as male because their sexual phenotype is male. (As we see later in the chapter, these XX males usually have a small piece of the Y chromosome that is attached to another chromosome.)

Gender is not the same as sex. Biological sex refers to the anatomical and physiological phenotype of an individual. Gender is a category assigned by the individual or others based on behavior and cultural practices. One's gender need not coincide with one's biological sex.

CONCEPTS

In sexual reproduction, two parents contribute genes to produce an offspring that is genetically distinct from both parents. In most eukaryotes, sexual reproduction consists of meiosis, which produces haploid gametes (or spores), and fertilization, which produces a diploid zygote.

✔ CONCEPT CHECK 1

What process causes the genetic variation seen in offspring produced by sexual reproduction?

As discussed in the introduction to this chapter, there are many ways in which sex differences arise. What are some of the different ways in which sex is determined in different organisms?

In some species, both sexes are present in the same organism, a condition termed **hermaphroditism**; organisms that bear both male and female reproductive structures are said to be **monoecious** (meaning "one house"). Species in which an individual organism has either male or female reproductive structures are said to be **dioecious** ("two houses"). Humans are dioecious. Among dioecious species, sex may be determined chromosomally, genetically, or environmentally.

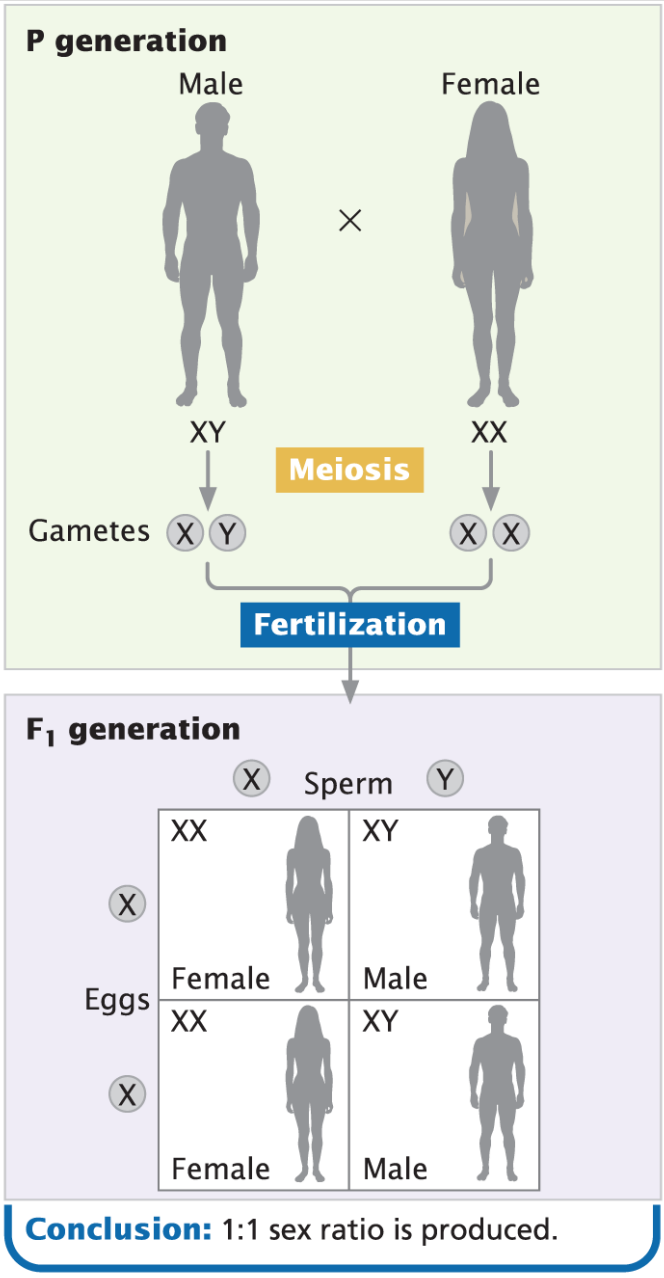
Chromosomal Sex-Determining Systems

The chromosome theory of heredity (see Chapter 3) states that genes are located on chromosomes, which serve as vehicles for the segregation of genes in meiosis. Definitive proof of this theory was provided by the discovery that the sex of certain insects is determined by the presence or absence of particular chromosomes.

In 1891, Hermann Henking noticed a peculiar structure in the nuclei of cells from male insects. Understanding neither its function nor its relation to sex, he called this structure the X body. Later, Clarence E. McClung studied the X body in grasshoppers and recognized that it was a chromosome. McClung called it the accessory chromosome, but it eventually became known as the X chromosome, from Henking's original designation. McClung observed that the cells of

female grasshoppers had one more chromosome than the cells of male grasshoppers, and he concluded that accessory chromosomes played a role in sex determination. In 1905, Nettie Stevens and Edmund Wilson demonstrated that in grasshoppers and other insects, the cells of females have two X chromosomes, whereas the cells of males have a single X. In some insects, they counted the same number of chromosomes in the cells of males and females but saw that one chromosome pair was different: two X chromosomes were found in female cells, whereas a single X chromosome plus a smaller chromosome, which they called Y, was found in male cells.

Stevens and Wilson also showed that the X and Y chromosomes separate into different cells in sperm formation: half of the sperm receive an X chromosome and the other half receive a Y. All eggs produced by the female in meiosis receive one X chromosome. A sperm containing a Y chromosome unites with an X-bearing egg to produce an XY male, whereas a sperm containing an X chromosome unites with an X-bearing egg to produce an XX female. This distribution of X and Y chromosomes in sperm accounts for the 1:1 sex ratio observed in most dioecious organisms (**Figure 4.5**). Because sex is inherited like other genetically determined characteristics, Stevens and Wilson's discovery that sex is associated with the inheritance of a particular chromosome also demonstrated that genes are located on chromosomes.



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4.5 Inheritance of sex in organisms with X and Y chromosomes results in equal numbers of male and female offspring.

Description

The illustration shows P generation where a male human with genotype big X big Y is crossed with a female human with genotype big X big X. Meiosis occurs and the gametes

produced are as follows: big X and big Y from male, big X and big X from female. Fertilization occurs and F subscript 1 generation is obtained. A Punnett square with two rows and two columns has the following entries:

The row headers represent eggs and are as follows: big X and big X.

The column headers represent sperm and are as follows: big X and big Y.

Row 1 column 1: big X big X (female)

Row 1 column 2: big X big Y (male)

Row 2 column 1: big X big X (female)

Row 2 column 2: big X big Y (male)

The conclusion states that 1 is to 1 sex ratio is produced.

As Stevens and Wilson found for insects, sex in many organisms is determined by a pair of chromosomes, the **sex chromosomes**, which differ between males and females. The nonsex chromosomes, which are the same for males and females, are called **autosomes**. We think of sex in organisms with sex chromosomes as being determined by the presence of the sex chromosomes, but in fact, the individual genes located on the sex chromosomes, in conjunction with the genes on the autosomes, are usually responsible for the sexual phenotypes.

XX-XO SEX DETERMINATION

The mechanism of sex determination in the grasshoppers studied by McClung, called the XX-XO system, is one of the simplest

mechanisms of chromosomal sex determination. In this system, females have two X chromosomes (XX) and males possess a single X chromosome (XO). There is no O chromosome—the letter O signifies the absence of a sex chromosome.

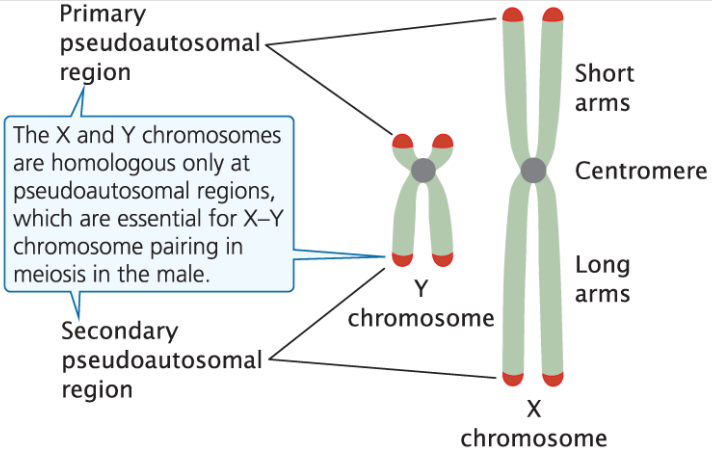
In meiosis in females, the two X chromosomes pair and then separate, and one X chromosome enters each haploid egg. In males, the single X chromosome segregates in meiosis to half the sperm cells; the other half receive no sex chromosome. Because males produce two different types of gametes with respect to the sex chromosomes, they are said to be the **heterogametic sex**. Females, which produce gametes that are all the same with respect to the sex chromosomes, are the **homogametic sex**. In the XX-XO system, the sex of an individual is therefore determined by which type of male gamete fertilizes the egg. X-bearing sperm unite with X-bearing eggs to produce XX zygotes, which develop into females. Sperm lacking an X chromosome unite with X-bearing eggs to produce XO zygotes, which develop into males.

XX-XY SEX DETERMINATION

In many species, the cells of males and females have the same number of chromosomes, but the cells of females have two X chromosomes (XX) and the cells of males have a single X chromosome and a smaller sex chromosome, the Y chromosome (XY). In humans and many other organisms, the Y chromosome is acrocentric (**Figure 4.6**), not Y shaped, as is often assumed. In this

sex-determining system, the male is the heterogametic sex—half of his gametes have an X chromosome and half have a Y chromosome. The female is the homogametic sex—all her eggs contain an X chromosome. Many organisms, including some plants, insects, and reptiles, and all mammals (including humans), have the XX-XY sex-determining system. Other organisms have variations of the XX-XY system; for example, the duck-billed platypus has an interesting system in which females have five pairs of X chromosomes and males have five pairs of X and Y chromosomes.

THINK-PAIR-SHARE Question 1



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4.6 The X and Y chromosomes in humans differ in size and genetic content. They are homologous only in the pseudoautosomal regions.

Description

The caption in the figure reads as follows: the X and Y chromosomes are homologous only at the pseudoautosomal regions, which are essential for X-Y chromosome pairing in meiosis in the male. In the drawing, the telomeric regions at the tips of the short arms of

the X and Y chromosomes are labeled the primary pseudoautosomal regions and are colored red. The telomeric regions at the tips of the long arms of the chromosomes are labeled the secondary pseudoautosomal regions and are also colored red. The short arm of Y chromosome is small as compared to its long arm and Y chromosome is shorter as compared to the X chromosome.

Although the X and Y chromosomes are not generally homologous, they do pair and segregate into different cells in meiosis. They can pair because these chromosomes are homologous in small regions called the **pseudoautosomal regions** (see Figure 4.6), in which they carry the same genes. In humans, there are pseudoautosomal regions at both tips of the X and Y chromosomes.

ZZ-ZW SEX DETERMINATION

In the ZZ-ZW sex-determining system, found in the bearded dragons discussed in the chapter introduction, the female is heterogametic and the male is homogametic. To prevent confusion with the XX-XY system, the sex chromosomes in this system are called Z and W, but the chromosomes do not resemble Zs and Ws. Females in this system are ZW; after meiosis, half of the eggs have a Z chromosome and the other half have a W chromosome. Males are ZZ; all sperm contain a single Z chromosome. The ZZ-ZW system is found in birds, some reptiles, butterflies, some amphibians, and some fishes.

THINK-PAIR-SHARE Question 2

CONCEPTS

The discovery that the presence or absence of particular chromosomes determines sex in insects provided evidence that genes are located on chromosomes. In XX-XO sex determination, the male is XO and heterogametic, and the female is XX and homogametic. In XX-XY sex determination, the male is XY and the female is XX; in this system, the male is heterogametic. In ZZ-ZW sex determination, the female is ZW and the male is ZZ; in this system, the female is the heterogametic sex.

✔ CONCEPT CHECK 2

How does the heterogametic sex differ from the homogametic sex?

a. The heterogametic sex is male; the homogametic sex is female.
b. Gametes of the heterogametic sex have different sex chromosomes; gametes of the homogametic sex all have the same sex chromosome.
c. Gametes of the heterogametic sex all contain a Y chromosome; gametes of the homogametic sex all contain an X chromosome.

Genic Sex Determination

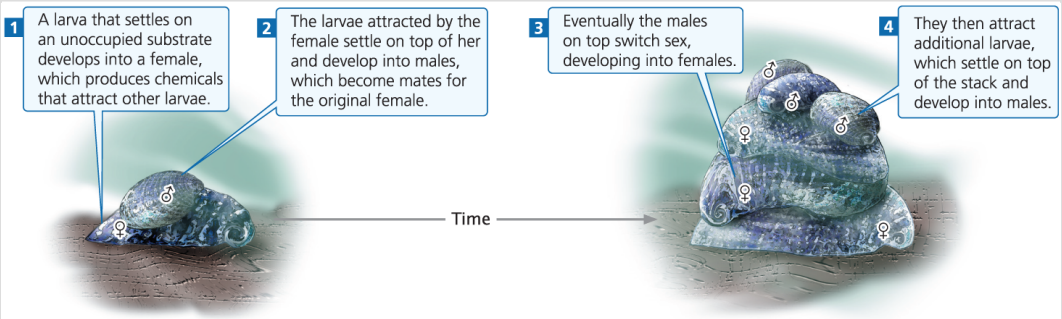
In some organisms, sex is genetically determined, but there are no obvious differences in the chromosomes of males and females: there are no sex chromosomes. These organisms have **genic sex determination**: genotypes at one or more loci determine the sex of an individual. Scientists have observed genic sex determination in some plants, fungi, protozoans, and fishes.

It is important to understand that even in chromosomal sex-determining systems, sex is actually determined by individual genes. In mammals, for example, a gene (*SRY*, discussed later in this chapter) located on the Y chromosome determines the male phenotype. In both genic sex determination and chromosomal sex determination, sex is controlled by individual genes; the difference is that with chromosomal sex determination, the sex chromosomes also look different in males and females.

Environmental Sex Determination

In a number of organisms, sex is determined fully or in part by environmental factors. A fascinating example of environmental sex determination is seen in the marine mollusk *Crepidula fornicata*, also known as the common slipper limpet (**Figure 4.7**). Slipper limpets live in stacks, one on top of another. Each limpet begins life as a swimming larva. The first larva to settle on a solid, unoccupied substrate develops into a female limpet. It then produces chemicals that attract other larvae, which settle on top of it. These larvae develop into males, which then serve as mates for the limpet below. After a period of time, the males on top develop into females and, in turn, attract additional larvae, which settle on top of the stack, develop into males, and serve as mates for the limpets under them. Limpets can form stacks of a dozen or more animals; the uppermost animals are always male. This type of sexual development is called **sequential hermaphroditism**; each individual animal can be both

male and female, although not at the same time. In *C. fornicata*, sex is determined environmentally by the limpet's position in the stack.



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4.7 In *Crepidula fornicata*, the common slipper limpet, sex is determined by an environmental factor—the limpet's position in the stack.

Description

The illustration shows the conversion of two slipper limpets into a larger stack of limpets with time. On the left side, two common slipper limpets are present. The first caption of four, corresponding to the rear bottom of the first pile, reads, "A larva that settles on an unoccupied substrate develops into a female, which produces chemicals that attract other larvae." The second caption, referring to the top of the pile, reads, "The larvae attracted by the female settle on top of her and develop into males, which become mates for the original female." A rightward arrow from the two slipper limpets to the pile of many indicates time and the slipper limpet converts to the stack of limpets on the right side. The third caption refers to the position of the original male on the stack of limpets, which is now occupied by a female, and reads, "Eventually the males on top switch sex developing into females." The fourth caption refers to the top of the stack and reads, "They then attract additional larvae, which settle on top of the stack and develop into males."

Environmental factors are also important in determining sex in some reptiles; the sexual phenotype of many turtles, crocodiles, alligators, and a few birds is affected by temperature during embryonic development. In some turtles, for example, warm incubation

temperatures produce more females, whereas cool temperatures produce males. In alligators, the reverse is true. In a few species, sex chromosomes determine whether individuals are male or female, but environmental factors can override this chromosomal sex determination. As discussed in the introduction to this chapter, bearded dragons are normally male when ZZ and female when ZW, but when the eggs are incubated at high temperatures, ZZ individuals develop into phenotypic females. Some of the different types of sex determination are summarized in Table 4.1.

TABLE 4.1 Some common sex-determining systems

System	Mechanism		Heterogametic Sex	Organisms
XX-XO	Females XX	Males X	Male	Some grasshoppers and other insects
XX-XY	Females XX	Males XY	Male	Many insects, fishes, amphibians, reptiles; mammals, including humans
ZZ-ZW	Females ZW	Males ZZ	Female	Butterflies, birds; some reptiles and amphibians
Genic sex determination	No distinct sex chromosomes Sex determined by genes on undifferentiated chromosomes		Varies	Some plants, fungi, protozoans, and fishes
Environmental sex determination	Sex determined by environmental factors		None	Some invertebrates, turtles, and alligators

Now that we have surveyed some of the different ways in which sex can be determined, we will examine one mechanism (the XX-XY system) in detail. Sex determination is XX-XY in both fruit flies and humans, but as we will see, the ways in which the X and Y chromosomes determine sex in these two organisms are quite different. ❯ **TRY PROBLEMS 4 AND 21**

CONCEPTS

In genic sex determination, sex is determined by genes at one or more loci, but there are no obvious differences in the chromosomes of males and females. In environmental sex determination, sex is determined fully or in part by environmental factors.

✔ CONCEPT CHECK 3

How do chromosomal, genic, and environmental sex-determining systems differ?

Sex Determination in *Drosophila melanogaster*

The fruit fly *Drosophila melanogaster* has eight chromosomes: three pairs of autosomes and one pair of sex chromosomes. Usually, females have two X chromosomes, and males have an X chromosome and a Y chromosome. In the 1920s, Calvin Bridges proposed that sex in *Drosophila* was determined not by the number of X and Y chromosomes but rather by the balance of female-

determining genes on the X chromosome and male-determining genes on the autosomes. He suggested that a fly's sex is determined by the so-called X : A ratio that is the number of X chromosomes divided by the number of haploid sets of autosomes. Normal flies possess two haploid sets of autosomes and either two X chromosomes (females) or one X chromosome and a Y chromosome (males). Bridges proposed that an X : A ratio of 1.0 produces a female fly and that an X : A ratio of 0.5 produces a male fly. He also suggested that an X : A ratio between 1.0 and 0.5 produces an intersex fly, with a mixture of male and female characteristics. An X : A ratio of less than 0.5 or greater than 1.0 produces developmentally abnormal flies called metamales and metafemales, respectively. When Bridges and others examined flies with different numbers of sex chromosomes and autosomes, the X : A ratio appeared to correctly predict the phenotypic sex of the flies (Table 4.2).

TABLE 4.2 Chromosome complements and sexual phenotypes in *Drosophila*

Sex-Chromosome Complement	Haploid Sets of Autosomes	X : A Ratio	Sexual Phenotype
XX	AA	1.0	Female
XY	AA	0.5	Male
XO	AA	0.5	Male

XXY	AA	1.0	Female
XXX	AA	1.5	Metafemale
XXXY	AA	1.5	Metafemale
XX	AAA	0.67	Intersex
XO	AAA	0.33	Metamale
XXXX	AAA	1.3	Metafemale

Although the X : A ratio correctly *predicts* the sexual phenotype, research now suggests that the *mechanism* of sex determination is not a balance between X-linked genes and autosomal genes, as Bridges proposed. Researchers have located a number of genes on the X chromosome that affect sexual phenotype, but few autosomal sex-determining genes (required for the X : A ratio hypothesis) have been identified. New evidence indicates that genes on the X chromosome are the primary sex determinant. The influence of the number of sets of autosomes on sex is indirect, affecting the timing of developmental events and therefore how long sex-determining genes on the X chromosome are active. For example, XX flies with three autosomal sets (XX, AAA) have an X : A ratio of 0.67 and develop an intersex phenotype. In these flies, the presence of three autosomal sets causes a critical developmental stage to shorten, not allowing female factors encoded on the X chromosomes enough

time to accumulate, with the result that the flies end up with an intersex phenotype. Thus, the number of autosomal sets of chromosomes influences sex determination in *Drosophila*, but not through the action of autosomal genes, as envisioned by Bridges.

CONCEPTS

Although the sexual phenotype of a fruit fly is predicted by the X : A ratio, sex is actually determined by genes on the X chromosome.

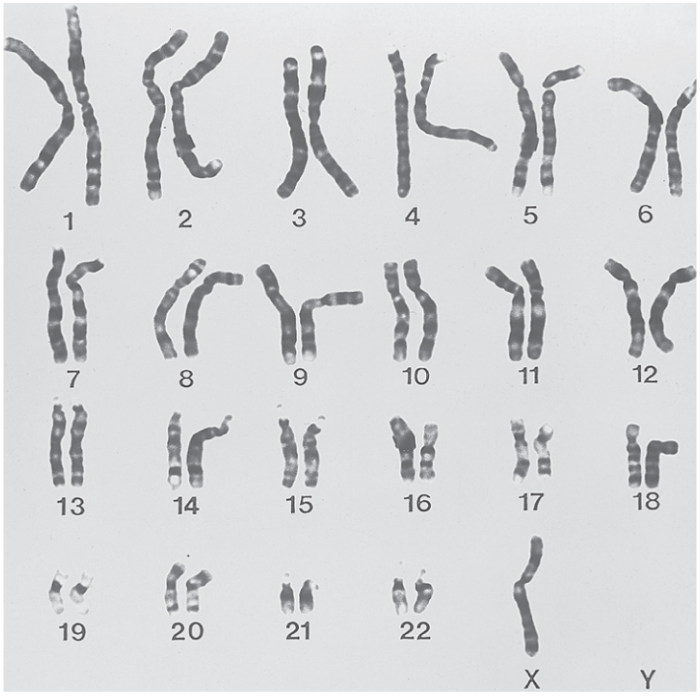
Sex Determination in Humans

Humans, like *Drosophila*, have XX-XY sex determination, but in humans, maleness is primarily determined by the presence of a particular gene (*SRY*) on the Y chromosome. The phenotypes that result from abnormal numbers of sex chromosomes, which arise when the sex chromosomes do not segregate properly in meiosis or mitosis, illustrate the importance of the Y chromosome in human sex determination.

TURNER SYNDROME

People who have **Turner syndrome** are female and often have underdeveloped secondary sex characteristics. This syndrome is

seen in 1 of 3000 female births. Affected women are frequently short and have a low hairline, a relatively broad chest, and folds of skin on the neck. Their intelligence is usually within the normal range. Many women who have Turner syndrome are sterile. In 1959, Charles Ford used new techniques to study human chromosomes and discovered that cells from a 14-year-old girl with Turner syndrome had only a single X chromosome (**Figure 4.8**); this chromosome complement is usually referred to as XO. Many people with Turner syndrome have some cells that are XX and other cells that are XO, a situation referred to as mosaicism (see p. 158).



Dept. of Clinical Cytogenetics, Addenbrookes Hospital/Science Photo Library/Science Source.

4.8 People with Turner syndrome have a single X chromosome in their cells.

Description

The illustration shows the pairs 1 through 18 are gradually decreasing in length. Pairs 19 through 22 are smaller in size. In the sex chromosome pair 23, only one X chromosome is present and the Y chromosome is missing indicating Turner syndrome.

There are no known cases in which a person is missing both X chromosomes, an indication that at least one X chromosome is necessary for human development. Presumably, embryos missing both Xs spontaneously abort in the early stages of development.

KLINEFELTER SYNDROME

People who have **Klinefelter syndrome**, which has a frequency of about 1 in 1000 male births, have cells with one or more Y chromosomes and multiple X chromosomes. The cells of most males with this condition are XXY (**Figure 4.9**), but the cells of a few males with Klinefelter syndrome are XXXY, XXXXY, or XXYY. Men with this condition frequently have small testes and reduced facial and pubic hair. They are often taller than normal and sterile; most have intelligence within the normal range.

Biophoto Associates/Science Source.

4.9 People with Klinefelter syndrome have a Y chromosome and two or more X chromosomes in their cells.

Description
The illustration shows both the chromosomes in a pair are roughly of same size. In the sex chromosome pair 23, two long X chromosomes are present along with a smaller Y chromosome indicating Klinefelter syndrome.

POLY-X FEMALES

In about 1 in 1000 female births, the infant's cells possess three X chromosomes, a condition often referred to as **triple-X syndrome**. These individuals have no distinctive features other than a tendency to be tall and thin. Although a few are sterile, many menstruate regularly and are fertile. The incidence of intellectual disability among triple-X females is slightly greater than that in the general population, but most XXX females have intelligence within the normal range. Much rarer are females whose cells contain four or

five X chromosomes. These females usually have normal female anatomy but are intellectually disabled and have a number of physical problems. The severity of intellectual disability increases as the number of X chromosomes increases beyond three.

XYY MALES

Males with an extra Y chromosome (XYY) occur with a frequency of about 1 in 1000 male births. These individuals have no distinctive physical characteristics other than a tendency to be several inches taller than the average for XY males. Their IQ is usually within the normal range; however, some studies suggest that learning difficulties may be more common in this group than in XY males.

THE ROLE OF SEX CHROMOSOMES

The phenotypes associated with sex-chromosome anomalies allow us to make several inferences about the role of sex chromosomes in human sex determination:

1. The X chromosome contains genetic information essential for both sexes; at least one copy of an X chromosome is required for human development.
2. The male-determining gene is located on the Y chromosome. A single copy of this chromosome, even in the presence of several X chromosomes, usually produces a male phenotype.

3. The absence of the Y chromosome usually results in a female phenotype.
4. Genes affecting fertility are located on the X and Y chromosomes. A female usually needs at least two copies of the X chromosome to be fertile.
5. Additional copies of the X chromosome may upset normal development in both males and females, producing physical problems and intellectual disabilities that increase as the number of extra X chromosomes increases.

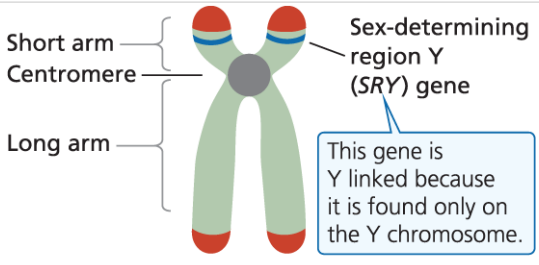
THE MALE-DETERMINING GENE IN HUMANS

The Y chromosome in humans, and in all other mammals, is of paramount importance in producing a male phenotype. However, scientists have discovered a few rare XX males whose cells apparently lack a Y chromosome. For many years, these males presented an enigma: How could a male phenotype exist without a Y chromosome? Close examination eventually revealed a small part of the Y chromosome attached to another chromosome, usually the X. This finding indicates that it is not the entire Y chromosome that determines maleness in humans; rather, it is a gene on the Y chromosome.

Early in development, all humans possess undifferentiated gonads and both male and female reproductive ducts. Then, about six weeks after fertilization, a gene on the Y chromosome becomes active. This gene causes the neutral gonads to develop into testes, which begin

to secrete two hormones: testosterone and anti-Müllerian hormone. Testosterone and other androgens induce the development of male characteristics, and anti-Müllerian hormone causes the degeneration of the female reproductive ducts. In the absence of this male-determining gene, the neutral gonads become ovaries, and female features develop. In mice, the regression of male reproductive ducts in females is promoted by another protein called chicken ovalbumin upstream promoter transcription factor 2 (COUP-TFII).

The male-determining gene in humans, called the **sex-determining region Y (*SRY*) gene**, was discovered in 1990 (**Figure 4.10**). This gene is found in XX males and is missing from rare XY females; it is also found on the Y chromosome of other mammals. Definitive proof that *SRY* is the male-determining gene came when scientists placed a copy of this gene in XX mice by means of genetic engineering. The XX mice that received this gene, although sterile, developed into anatomical males.



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4.10 The *SRY* gene, located on the Y chromosome, causes the development of male characteristics.

Description
The illustration shows an X-shaped Y chromosome. Centromere is labeled as the point where the chromatids are joined. The short arm is the region above the centromere and long arm is the region below the centromere. The short arm is relatively shorter than the long arm. Two bands representing sex-determining region, or S R Y gene are labeled on the short arms of both the chromatids as thin blue bands on each side. The tips of both the chromatids are colored red. A caption reads, the S R Y gene is Y-linked because it is found only on the Y chromosome.

The *SRY* gene encodes a protein called a transcription factor (see Chapter 13), which binds to DNA and stimulates the transcription of genes that promote the differentiation of the testes. Although *SRY* is the primary determinant of maleness in humans, a number of other genes (some X linked, others Y linked, and still others autosomal) also have roles in fertility and the development of sexual phenotypes.

THINK-PAIR-SHARE Question 3

CONCEPTS

The presence of the *SRY* gene on the Y chromosome causes a human embryo to develop as a male. In the absence of this gene, a human embryo develops as a female.

✔ CONCEPT CHECK 4

What is the phenotype of a person who has XXXY sex chromosomes?

a. Klinefelter syndrome

b. Turner syndrome
c. Poly-X female

ANDROGEN-INSENSITIVITY SYNDROME

Although the *SRY* gene is the primary determinant of sex in human embryos, several other genes influence sexual development, as illustrated by women with androgen-insensitivity syndrome. These individuals have female external sexual characteristics. Indeed, most are unaware of their condition until they reach puberty and fail to menstruate. Examination by a gynecologist reveals that the vagina ends blindly and that the uterus, oviducts, and ovaries are absent. Inside the abdominal cavity, a pair of testes produce levels of testosterone normally seen in males. The cells of a woman with androgen-insensitivity syndrome contain an X and a Y chromosome.

How can a person be female in appearance when her cells contain a Y chromosome and she has testes that produce testosterone? The answer lies in the complex relation between genes and sex in humans. In a human embryo with a Y chromosome, the *SRY* gene causes the gonads to develop into testes, which produce testosterone. Normally, testosterone stimulates embryonic tissues to develop male characteristics. But for testosterone to have its effect, it must bind to an androgen receptor. This receptor is defective in females with androgen-insensitivity syndrome; consequently, their cells do not respond to testosterone, and female characteristics

develop. The gene for the androgen receptor is located on the X chromosome, so people with this condition always inherit it from their mothers.

Androgen-insensitivity syndrome illustrates several points about the influence of genes on a person's sex. First, this condition demonstrates that human sexual development is a complex process, influenced not only by the *SRY* gene on the Y chromosome but also by other genes found elsewhere. Second, it shows that most people carry genes for both male and female characteristics, as illustrated by the fact that those with androgen-insensitivity syndrome have the capacity to develop female characteristics even though they have a Y chromosome. Indeed, the genes for most male and female secondary sex characteristics are present not on the sex chromosomes but on the autosomes. The key to maleness and femaleness lies not in the genes but in the control of their expression. › **TRY PROBLEM 17**

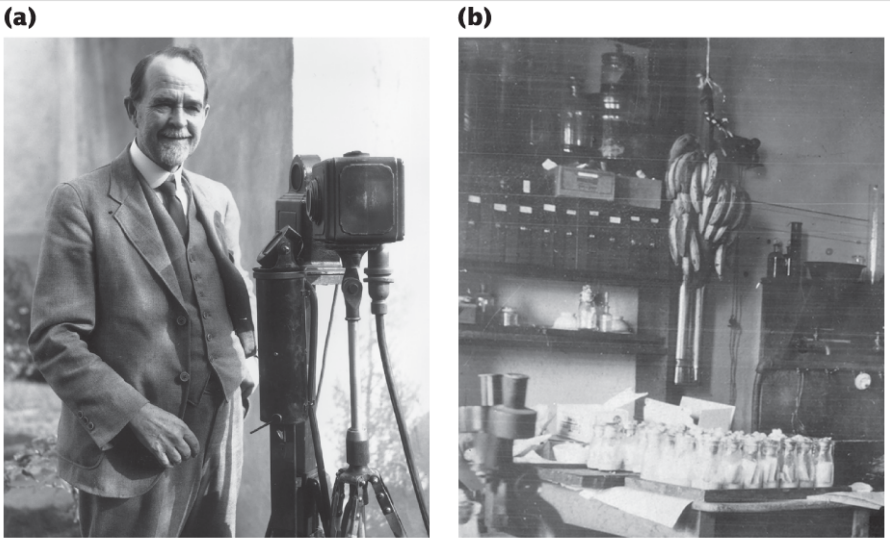
4.2 Sex-Linked Characteristics Are Determined by Genes on the Sex Chromosomes

In Chapter 3, we learned several basic principles of heredity that Mendel discovered in his experiments with pea plants. A major extension of these Mendelian principles is the pattern of inheritance exhibited by **sex-linked characteristics**: characteristics determined by genes located on the sex chromosomes. Genes on the X chromosome determine **X-linked characteristics**; those on the Y chromosome determine **Y-linked characteristics**. Because the Y chromosome of many organisms contains little genetic information, most sex-linked characteristics are X linked. Males and females differ in their sex chromosomes, so the pattern of inheritance for sex-linked characteristics differs from that exhibited by genes located on autosomes.

X-Linked White Eyes in *Drosophila*

The first person to provide an explanation for sex-linked inheritance was the American biologist Thomas Hunt Morgan (**Figure 4.11**). Morgan began his career as an embryologist, but the discovery of Mendel's principles inspired him to begin conducting genetic experiments, initially on mice and rats. In 1909, Morgan switched his

research to *Drosophila melanogaster*; a year later, he discovered among the flies of his laboratory colony a single male that possessed white eyes, in stark contrast to the red eyes of normal fruit flies. This fly had a tremendous effect on Morgan's career as a biologist and on the future of genetics.



Part a: Associated Press. Part b: American Philosophical Society.

4.11 Thomas Hunt Morgan's work with *Drosophila* helped unravel many basic principles of genetics, including X-linked inheritance. (a) Morgan. (b) The Fly Room, where Morgan and his students conducted genetic research.

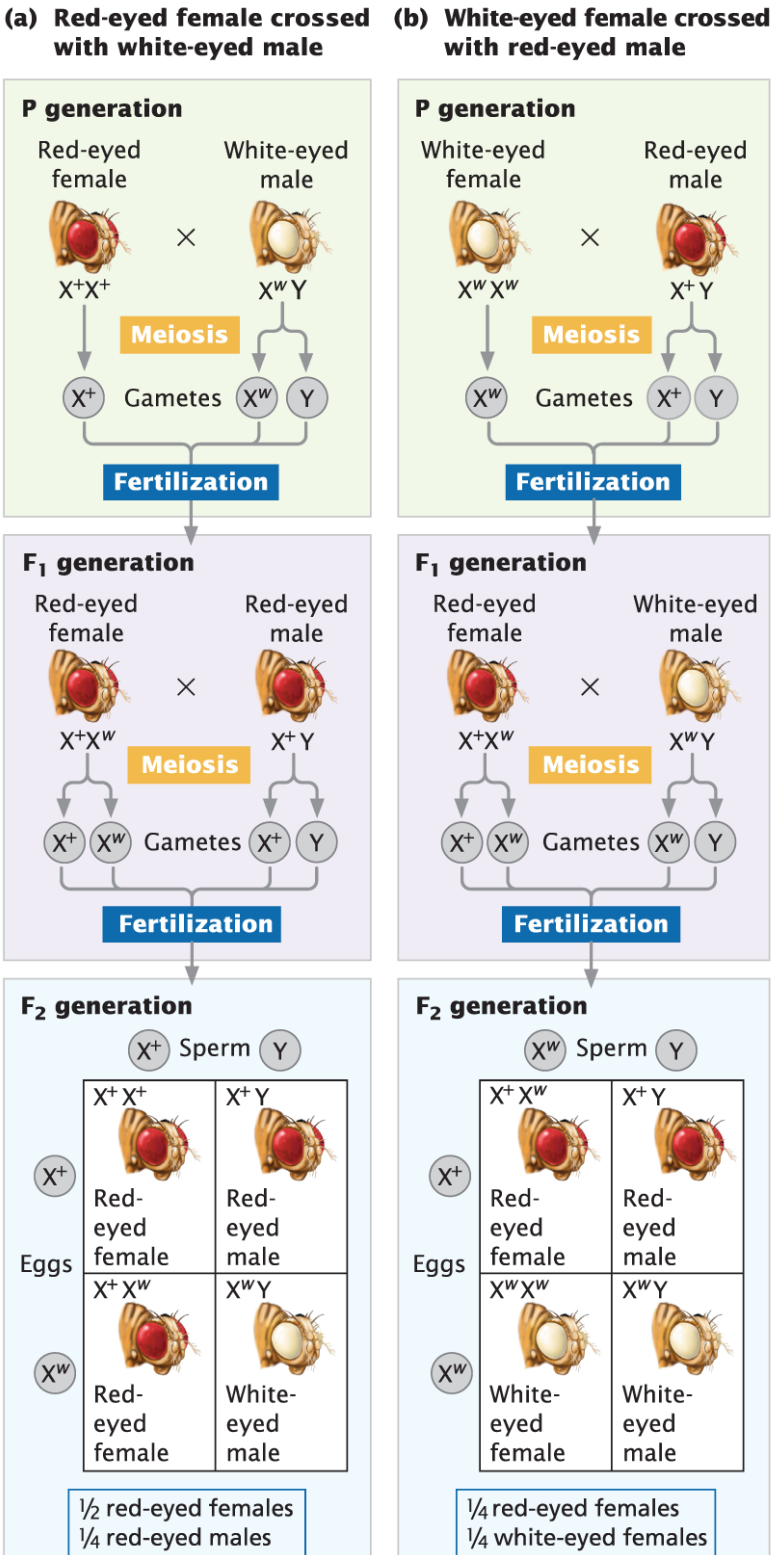
To investigate the inheritance of the white-eye trait in fruit flies, Morgan systematically carried out a series of genetic crosses. First, he crossed pure-breeding red-eyed females with his white-eyed male, producing F_1 progeny that had red eyes (Figure 4.12a). Morgan's results from this initial cross were consistent with Mendel's principles: a cross between a homozygous dominant individual and a homozygous recessive individual produced heterozygous offspring exhibiting the dominant trait. These results suggested that white

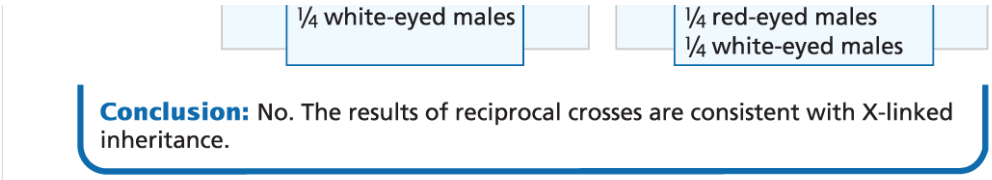
eyes are a simple recessive trait. When Morgan crossed the F_1 flies with each other, however, he found that all the female F_2 flies possessed red eyes, but half the male F_2 flies had red eyes and the other half had white eyes. This finding was clearly not the expected result for a simple recessive trait, which should appear in one-fourth of both male and female F_2 offspring.

Experiment

Question: Are white eyes in fruit flies inherited as an autosomal recessive trait?

Methods Perform reciprocal crosses.





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4.12 Morgan's crosses demonstrated the X-linked inheritance of white eyes in fruit flies. (a) Original and F_1 crosses. (b) Reciprocal crosses.

Description

The experiment question is: Are white eyes in fruit flies inherited as an autosomal recessive trait? The Methods involve Perform reciprocal crosses. Illustration "a" shows a cross between a red-eyed female and a white-eyed male. The P generation has a red-eyed female with genotype big X superscript positive big X superscript positive crossed with a white-eyed male with genotype big X superscript W big Y. Meiosis occurs and the gametes produced are as follows: big X superscript positive from the female and big X superscript W and big Y from the male. Fertilization occurs and the results show F subscript 1 generation. The F subscript 1 generation has a red-eyed female with genotype big X superscript positive big X superscript W crossed with a red-eyed male with genotype big X superscript positive big Y. Meiosis occurs and gametes produced are as follows: big X superscript positive and big X superscript W from the female and big X superscript positive and big Y from the male. Fertilization occurs and the results show the F subscript 2 generation. A Punnett square with 2 rows and 2 columns shows the following entries:

The column headers represent sperms and are as follows: big X superscript positive and big Y.

The row headers represent eggs and are as follows: big X superscript positive and big X superscript W.

Row 1 column 1: big X superscript positive big X superscript positive (red-eyed female)

Row 1 column 2: big X superscript positive big Y (red-eyed male)

Row 2 column 1: big X superscript positive big X superscript W (red-eyed female)

Row 2 column 2: big X superscript W big Y (white-eyed male)

Text below reads, “One-half red-eyed females, one-fourth red-eyed males, and one-fourth white eyed males.”

The illustration “b” shows a cross between a white-eyed female and a red-eyed male. The P generation has a white-eyed female with genotype big X superscript W big X superscript W crossed with a red-eyed male with genotype big X superscript positive big Y. Meiosis occurs and the gametes produced are as follows: big X superscript W from the female and big X superscript positive and big Y from the male. Fertilization occurs and the results show F subscript 1 generation. The F subscript 1 generation has a red-eyed female with genotype big X superscript positive big X superscript W crossed with a white-eyed male with genotype big X superscript W big Y. Meiosis occurs and the gametes produced are as follows: big X superscript positive and big X superscript W from the female and big X superscript positive and big Y from the male. Fertilization occurs and the results show the F subscript 2 generation. A Punnett square with 2 rows and 2 columns has the following entries.

The column headers represent sperms and are as follows: big X superscript W and big Y.

The row headers represent eggs and are as follows: big X superscript positive and big X superscript W.

Row 1 column 1: big X superscript positive big X superscript W (red-eyed female)

Row 1 column 2: big X superscript positive big Y (red-eyed male)

Row 2 column 1: big X superscript W big X superscript W (white-eyed female)

Row 2 column 2: big X superscript W big Y (white-eyed male)

Text below reads, “One-fourth red-eyed females, one-fourth white eyed females, one-fourth red-eyed males, and one-fourth white-eyed males.”

The conclusion states that no, the results of reciprocal crosses are consistent with X-linked inheritance.

To explain this unexpected result, Morgan proposed that the locus affecting eye color is on the X chromosome (i.e., that eye color is X

linked). He also recognized that eye-color alleles are present on the X chromosome only; no homologous allele is present on the Y chromosome. Because the cells of females possess two X chromosomes, females can be homozygous or heterozygous for the eye-color alleles. The cells of males, on the other hand, possess only a single X chromosome and can carry only a single eye-color allele. Males, therefore, cannot be homozygous or heterozygous but are said to be **hemizygous** for X-linked loci.

To verify his hypothesis that the white-eye trait is X linked, Morgan conducted additional crosses. He predicted that a cross between a white-eyed female and a red-eyed male would produce all red-eyed females and all white-eyed males (**Figure 4.12b**). When Morgan performed this cross, the results were exactly as predicted. Note that this cross is the reciprocal of the original cross and that the two reciprocal crosses produced different results in the F_1 and F_2 generations. Morgan also crossed the F_1 heterozygous females with their white-eyed father, the red-eyed F_2 females with white-eyed males, and white-eyed females with white-eyed males. In all these crosses, the results were consistent with Morgan's conclusion that the white-eye trait is an X-linked characteristic. You can view the results of Morgan's crosses in (A) **Animation 4.1**.

Nondisjunction and the Chromosome Theory of Inheritance

When Morgan crossed his original white-eyed male with homozygous red-eyed females, all 1237 of the progeny had red eyes except for 3 white-eyed males. Morgan attributed these white-eyed F_1 males to the occurrence of further random mutations. However, flies with these unexpected phenotypes continued to appear in his crosses. Although uncommon, they appeared far too often to be due to spontaneous mutation. Calvin Bridges, who was one of Morgan's students, set out to investigate the genetic basis of these exceptions.

Bridges found that the exceptions arose only in certain strains of white-eyed flies. When he crossed a white-eyed female from one of those strains with a red-eyed male, about 5% of the male offspring had red eyes and about 5% of the female offspring had white eyes. In this cross, the expected result is that every male fly should inherit its mother's X chromosome and should have the genotype X^wY and white eyes. Furthermore, every female fly should inherit a dominant red-eye allele on its father's X chromosome, along with a white-eye allele on its mother's X chromosome; thus, all the female progeny should be X^+X^w and have red eyes (see the F_1 progeny in Figure 4.12b). The continual appearance of red-eyed males and white-eyed females in this cross was therefore unexpected.

BRIDGES'S EXPLANATION

To explain the appearance of red-eyed males and white-eyed females in his cross, Bridges hypothesized that the exceptional white-eyed females of this strain actually possessed two X chromosomes and a

Y chromosome (X^wX^wY). In *Drosophila*, flies with XXY sex chromosomes normally develop as females, in spite of possessing a Y chromosome (see Table 4.2). About 90% of the time, the two X chromosomes of the X^wX^wY females separate from each other in anaphase I of meiosis, with an X and a Y chromosome entering one gamete and a single X entering another gamete (**Figure 4.13**). When these gametes are fertilized by sperm from a normal red-eyed male, white-eyed males and red-eyed females are produced. About 10% of the time, the two X chromosomes in the females fail to separate in anaphase I of meiosis, a phenomenon known as **nondisjunction**. When nondisjunction of the Xs occurs, half of the eggs receive two copies of the X chromosome and the other half receive only a Y chromosome (see Figure 4.13). When these eggs are fertilized by sperm from a normal red-eyed male, four combinations of sex chromosomes are produced. An egg with two X chromosomes that is fertilized by an X-bearing sperm produces an $X^+X^wX^w$ zygote, which usually dies. When an egg carrying two X chromosomes is fertilized by a Y-bearing sperm, the resulting zygote is X^wX^wY, which develops into a white-eyed female. An egg with only a Y chromosome that is fertilized by an X-bearing sperm produces an X^+Y zygote, which develops into a normal red-eyed male. If the egg with only a Y chromosome is fertilized by a Y-bearing sperm, the resulting zygote has two Y chromosomes and no X chromosome, and dies. Nondisjunction of the X chromosomes among X^wX^wY white-eyed females therefore produces a few white-eyed females and red-eyed males, which is exactly what Bridges found in his crosses. The

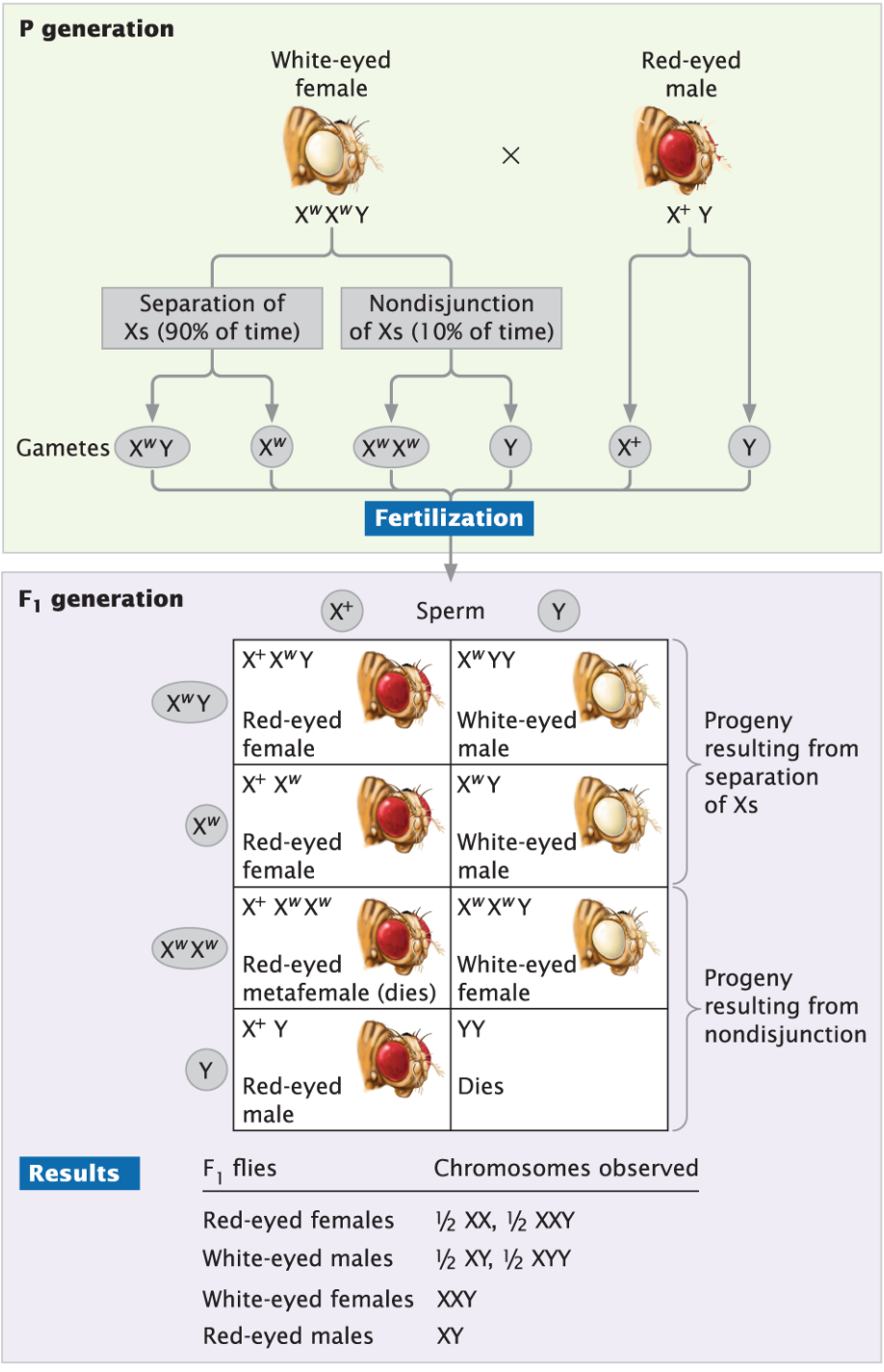
results of Bridges's crosses are further explained in **Animation 4.1**.

Experiment

Question: In a cross between a white-eyed female and a red-eyed male, why are a few white-eyed females and red-eyed males produced?

Methods

Hypothesis: White-eyed females and red-eyed males in F_1 result from nondisjunction in an XXY female.



Results

F_1 flies	Chromosomes observed
Red-eyed females	½ XX, ½ XXY
White-eyed males	½ XY, ½ XYY
White-eyed females	XXY
Red-eyed males	XY

Conclusion: The white-eyed females and red-eyed males in the F_1 result from nondisjunction of the X chromosomes in an XXY female.

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4.13 Bridges's crosses proved that the gene for white eyes is located on the X chromosome.

Description

The experiment question is: In a cross between a white-eyed female and a red-eyed male, why are a few white-eyed females and red-eyed males produced? The methods below read, Hypothesis: White-eyed females and red-eyed males in F subscript 1 result from nondisjunction in an big X big X big Y female.

The illustration shows P generation where a white-eyed female with genotype big X superscript W big X superscript W big Y is crossed with a red-eyed male with genotype big X superscript positive big Y. Gametes are produced from the female in two ways. Separation of X's (90 percent of time) produces the following gametes: big X superscript W big Y and big X superscript W. Non-disjunction of X's (10 percent of time) produces the following gametes: big X superscript W big X superscript W and big Y. Gametes produced from the male fly are as follows: big X superscript positive and big Y. Fertilization occurs and F subscript 1 generation is obtained. A Punnett square with 4 rows and 2 columns has the following entries:

The row headers are as follows: 1, big X superscript W big Y; 2, big X superscript W; 3, big X superscript W big X superscript W; and 4, big Y.

The column headers represent sperms and are as follows: big X superscript positive and big Y.

The following progenies result from separation of X's:

Row 1 column 1: big X superscript positive big X superscript W big Y (Red-eyed Female)

Row 1 column 2: big X superscript W big Y big Y (White-eyed Male)

Row 2 column 1: big X superscript positive big X superscript W (Red-eyed Female)

Row 2 column 2: big X superscript W big Y (White-eyed Male)

The following progenies result from non-disjunction:

Row 3 column 1: big X superscript positive big X superscript W big X superscript W (Red-eyed metafemale (dies))

Row 3 column 2: big X superscript W big X superscript W big Y (White-eyed Female)

Row 4 column 1: big X superscript positive big Y (Red-eyed Male)

Row 4 column 2: big Y big Y (dies)

The results below show the chromosomes observed in F subscript 1 flies as follows:

Red-eyed females; chromosomes observed, one-half big X big X and one-half big X big X big Y.

White-eyed males; chromosomes observed, one-half big X big Y and one-half big X big Y big Y.

White-eyed females; chromosomes observed, big X big X big Y.

Red-eyed males; chromosomes observed, big X big Y.

The conclusion states that the white-eyed females and red-eyed males in the F subscript 1 result from nondisjunction of the X chromosomes in a big X big X big Y female.

CONFIRMATION OF BRIDGES'S HYPOTHESIS

Bridges's hypothesis predicted that the white-eyed females from his crosses would possess two X chromosomes and one Y chromosome and that the red-eyed males would possess a single X chromosome. To verify his hypothesis, Bridges examined the chromosomes of his flies and found precisely what he had predicted. The significance of Bridges's study is not that it explained the appearance of an occasional odd fly in his colony but that he was able to link the inheritance of a specific gene (*w*) to the presence of a specific

chromosome (X). This association between genotype and chromosomes provided unequivocal evidence that sex-linked genes are located on the X chromosome and confirmed the chromosome theory of inheritance. ❯ **TRY** PROBLEM 22

CONCEPTS

By showing that the appearance of rare phenotypes is associated with the inheritance of particular chromosomes, Bridges proved that sex-linked genes are located on the X chromosome and that the chromosome theory of inheritance is correct.

✔ CONCEPT CHECK 5

What was the genotype of the few F_1 red-eyed males obtained by Bridges when he crossed a white-eyed female with a red-eyed male?

a. X^+
b. X^wX^+Y
c. X^+Y
d. X^+X^+Y

X-Linked Color Blindness in Humans

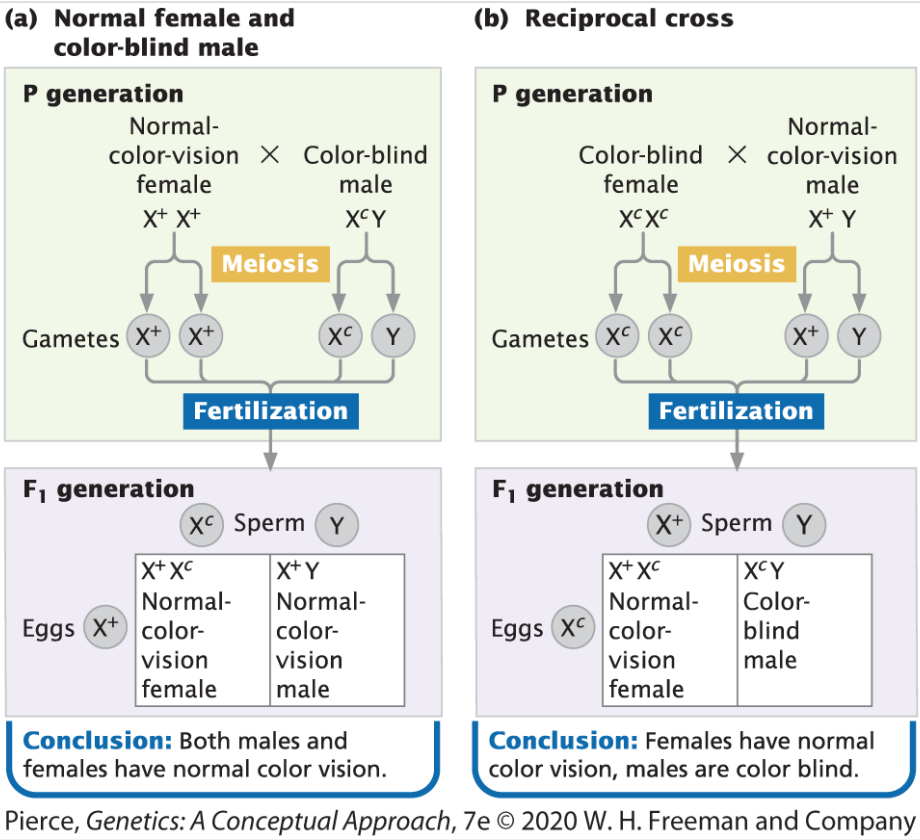
To further examine X-linked inheritance, let's consider another X-linked characteristic: red–green color blindness in humans. The human eye perceives color through light-sensing cone cells that line the retina. Each cone cell contains one of three pigments capable of

absorbing light of a particular wavelength: one absorbs blue light, a second absorbs red light, and a third absorbs green light. The human eye actually detects only three colors—blue, red, and green—but the brain mixes the signals from different cone cells to create the wide spectrum of colors that we perceive. Each of the three pigments is encoded by a separate locus; the locus for the blue pigment is found on chromosome 7, and those for the green and the red pigments lie close together on the X chromosome.

The most common types of human color blindness are caused by defects of the red and green pigments; we will refer to these conditions as red–green color blindness. Mutations that produce defective color vision are generally recessive, and because the genes encoding the red and the green pigments are located on the X chromosome, red–green color blindness is inherited as an X-linked recessive trait. (See Chapter 8 for additional information about the genes that cause red–green color blindness.)

We will use the symbol X^c to represent an allele for red–green color blindness and the symbol X^+ to represent an allele for normal color vision. Females possess two X chromosomes, so there are three possible genotypes among females: X^+X^+ and X^+X^c, which produce normal color vision, and X^cX^c, which produces color blindness. Males have only a single X chromosome and two possible genotypes: X^+Y, which produces normal color vision, and X^cY, which produces color blindness.

Let's consider what happens when a woman homozygous for normal color vision mates with a color-blind man (Figure 4.14a). All the gametes produced by the woman contain an allele for normal color vision. Half of the man's gametes receive the X chromosome, with the color-blindness allele, and the other half receive the Y chromosome, which carries no alleles affecting color vision. When an X^c-bearing sperm unites with the X^+-bearing egg, a heterozygous female with normal color vision (X^+X^c) is produced. When a Y-bearing sperm unites with the X^+-bearing egg, a hemizygous male with normal color vision (X^+Y) is produced.



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4.14 Red–green color blindness is inherited as an X-linked recessive trait in humans.

Description

The illustration “a” shows a normal female crossed with a color-blind male. The P generation has a normal color-vision female with genotype big X superscript positive big X superscript positive crossed with a color-blind male with genotype big X superscript C big Y. Meiosis occurs and the following gametes are produced: big X superscript positive and big X superscript positive from the female, big X superscript C and big Y from the male. Fertilization occurs and F subscript 1 generation is obtained. A Punnett square with 1 row and 2 columns shows the following data:

The column headers represent sperm and are as follows: big X superscript C and big Y.

The row header represents eggs and is as follows: big X superscript positive.

Row 1 column 1: big X superscript positive big X superscript C (Normal color vision

Female)

Row 1 column 2: big X superscript positive big Y (Normal color vision

Male).

A caption below reads, Conclusion: Both males and females have normal color vision.

Illustration “b” shows the reciprocal cross. The P generation has a color-blind female with genotype big X superscript C big X superscript C crossed with a normal color- vision male with genotype big X superscript positive big Y. Meiosis occurs and the gametes produced are as follows: big X superscript C, big X superscript C, big X superscript positive, and big Y. Fertilization occurs and F subscript 1 generation is obtained.

A Punnett square with 1 row and 2 columns shows the following:

The column headers represent sperm and are as follows: big X superscript positive and big Y.

The row header represents eggs and is as follows: big X superscript C.

Row 1 column 1: big X superscript positive big X superscript C (Normal color vision

Female)

Row 1 column 2: big X superscript C big Y (Color blind Male).

The conclusion states that females have normal color vision, males are color blind.

Now consider the reciprocal cross between a color-blind woman and a man with normal color vision (**Figure 4.14b**). The woman produces only X^c-bearing gametes. The man produces some gametes that contain the X chromosome and others that contain the Y chromosome. Males inherit the X chromosome from their mothers, so because both of the mother's X chromosomes bear the X^c allele, all the male offspring of this cross will be color blind. In contrast, females inherit an X chromosome from each parent; thus, all the female offspring of this cross will be heterozygous with normal color vision. A female is color blind only when she inherits color-blindness alleles from both parents, whereas a male need only inherit a color-blindness allele from his mother to be color blind; for this reason, color blindness and most other rare X-linked recessive traits are more common in males than in females.

In these crosses for color blindness, notice that an affected woman passes the X-linked recessive trait to her sons but not to her daughters, whereas an affected man passes the trait to his grandsons through his daughters but never to his sons. X-linked recessive characteristics may therefore appear to alternate between the sexes, appearing in females in one generation and in males in the next generation.

Recall that the X and Y chromosomes pair in meiosis because they are homologous at the small pseudoautosomal regions. Genes in

these regions of the X and Y chromosomes are homologous, just like those on autosomes, and they exhibit autosomal patterns of inheritance rather than the sex-linked inheritance seen for most genes on the X and Y chromosomes.

THINK-PAIR-SHARE Questions 4 and 5

WORKED PROBLEM

Now that we understand the pattern of X-linked inheritance, let's apply our knowledge to answer a specific question.

Red–green color blindness is an X-linked recessive trait. Betty has normal color vision, but her mother is color blind. Bill is color blind. If Bill and Betty have a child together, what is the probability that the child will be color blind?

Solution Strategy

What information is required in your answer to the problem?

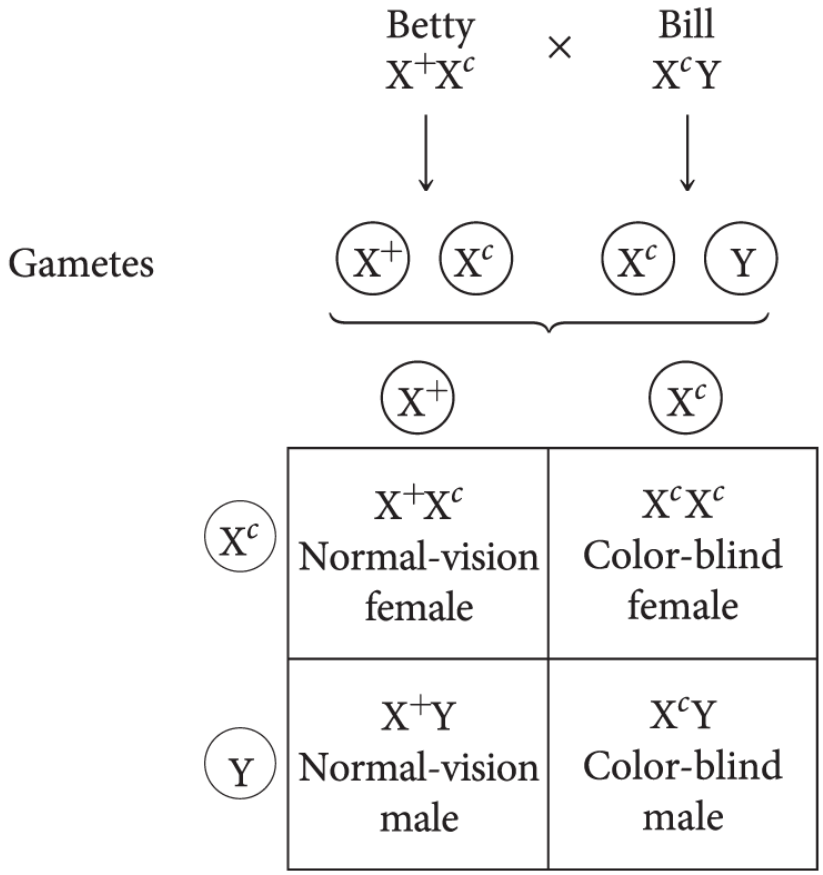
The probability that Bill and Betty's child will be color blind.

What information is provided to solve the problem?

Red–green color blindness is an X-linked recessive trait. The phenotypes of Betty, Betty's mother, and Bill.

Solution Steps

Because color blindness is an X-linked recessive trait, Betty's color-blind mother must be homozygous for the color-blindness allele (X^cX^c). Females inherit one X chromosome from each of their parents, so Betty must have inherited a color-blindness allele from her mother. Because Betty has normal color vision, she must have inherited an allele for normal color vision (X^+) from her father; thus, Betty is heterozygous (X^+X^c). Bill is color blind. Because males are hemizygous for X-linked alleles, he must be (X^cY). A mating between Betty and Bill is represented as



Description

Betty has genotype big X superscript positive big X superscript C and the gametes produced are as follows: big X superscript positive and big X superscript C. Bill has genotype big X superscript C big Y and the gametes produced are as follows: big X superscript C and big Y. A Punnett square with 2 rows and 2 columns has the following entries:

The column headers are as follows: big X superscript positive and big X superscript C

The row headers are as follows: big X superscript C and big Y

Row 1 column 1: big X superscript positive big X superscript C (Normal-vision

Female)

Row 1 column 2: big X superscript C big X superscript C (Color-blind Female)

Row 2 column 1: big X superscript positive big Y (Normal-vision Male)

Row 2 column 2: big X superscript C big Y (Color-blind Male)

Thus, the overall probability that their child will be color blind is $1/2$.

THINK-PAIR-SHARE Question 6

❯❯ Get some additional practice with X-linked inheritance by working **Problem 24** at the end of this chapter.

CONCEPTS

Characteristics determined by genes on the sex chromosomes are called sex-linked characteristics. In organisms with XX-XY sex determination, diploid females have two alleles at each X-linked locus, whereas diploid males possess a single allele at each X-linked locus. Females inherit X-linked alleles from both parents, but males inherit a single X-linked allele from their mothers.

✔ CONCEPT CHECK 6

Hemophilia (reduced blood clotting) is an X-linked recessive disease in humans. A woman with hemophilia mates with a man who exhibits normal blood clotting. What is the probability that their child will have hemophilia?

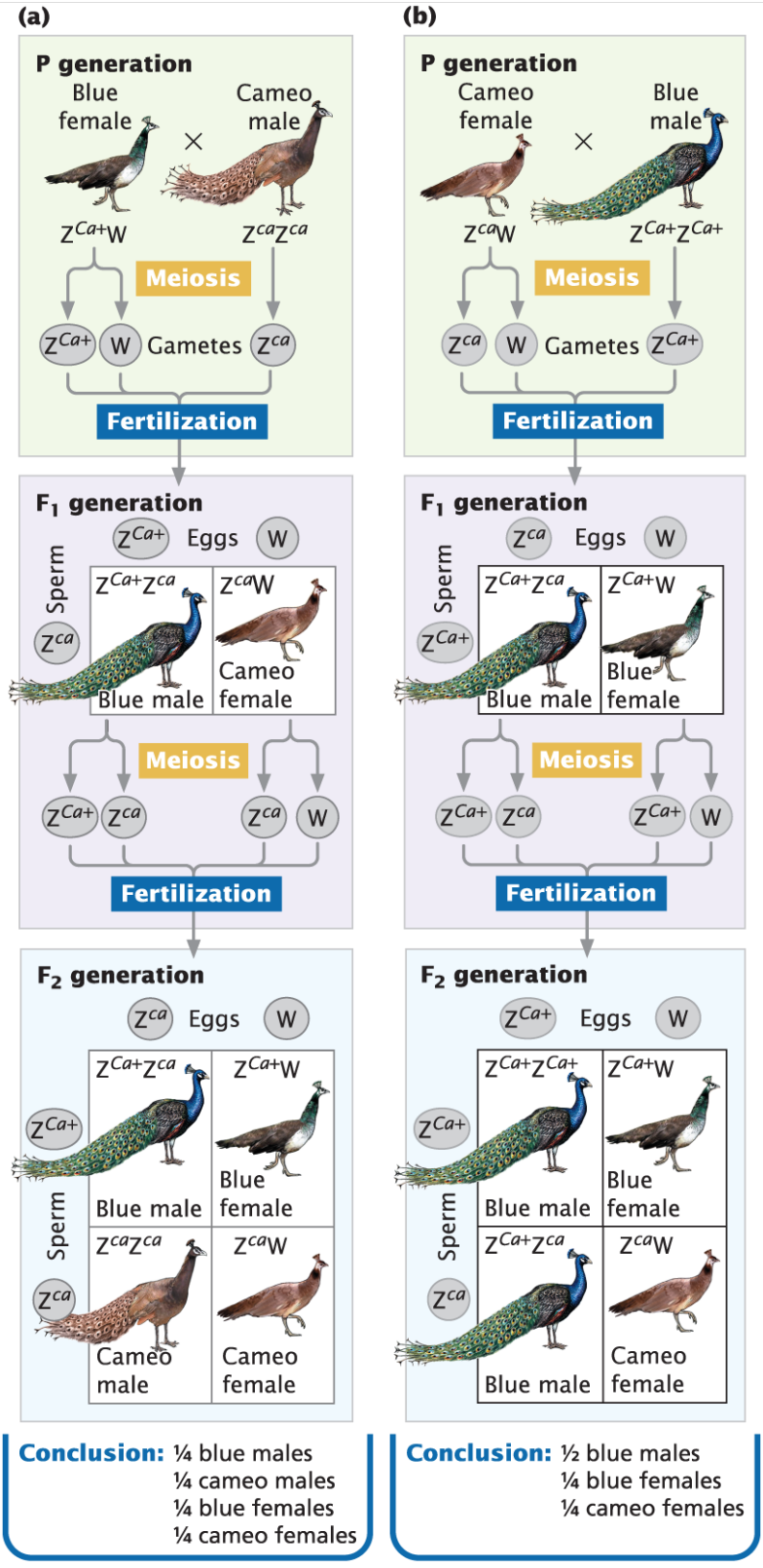
Symbols for X-Linked Genes

There are several different ways to record genotypes for X-linked characteristics. Sometimes these genotypes are written in the same way as those for autosomal characteristics. In this case, hemizygous males are simply given a single allele: for example, the genotype of a female *Drosophila* with white eyes is *ww*, and the genotype of a white-eyed hemizygous male is *w*. Another method is to include the Y chromosome, designating it with a diagonal slash (/). With this method, the white-eyed female's genotype is still *ww*, and the white-eyed male's genotype is *w*/. Perhaps the most useful method is to record the X and Y chromosomes in the genotype, designating the X-linked alleles with superscripts, as is done in this chapter. With this method, a white-eyed female is X^wX^w and a white-eyed male is X^wY. The use of Xs and Ys in the genotype has the advantage of reminding us that the genes are X linked and that the male must always have a single allele, inherited from the mother.

Z-Linked Characteristics

In organisms with ZZ-ZW sex determination, males are the homogametic sex (ZZ) and carry two sex-linked (usually referred to as Z-linked) alleles; thus, males may be homozygous or heterozygous. Females are the heterogametic sex (ZW) and possess only a single Z-linked allele. The inheritance of Z-linked characteristics is the same as that of X-linked characteristics, except that the pattern of inheritance in males and females is reversed.

One example of a Z-linked characteristic is the cameo phenotype in Indian blue peafowl (*Pavo cristatus*). In these birds, the wild-type plumage is a glossy metallic blue. The female peafowl is ZW and the male is ZZ. Cameo plumage, which produces brown feathers, results from a Z-linked allele (Z^{ca}) that is recessive to the wild-type blue allele (Z^{Ca+}). If a blue female ($Z^{Ca+}W$) is crossed with a cameo male ($Z^{ca}Z^{ca}$), all the F_1 females are cameo ($Z^{ca}W$) and all the F_1 males are blue ($Z^{Ca+}Z^{ca}$), as shown in **Figure 4.15**. When the F_1 are interbred, $^1/_4$ of the F_1 are blue males ($Z^{Ca+}Z^{ca}$), $^1/_4$ are blue females ($Z^{Ca+}W$), $^1/_4$ are cameo males ($Z^{ca}Z^{ca}$), and $^1/_4$ are cameo females ($Z^{ca}W$). The reciprocal cross of a cameo female with a homozygous blue male produces an F_1 generation in which all offspring are blue and an F_2 generation consisting of $^1/_2$ blue males ($Z^{Ca+}Z^{Ca+}$ and $Z^{Ca+}Z^{ca}$), $^1/_4$ blue females ($Z^{Ca+}W$), and $^1/_4$ cameo females ($Z^{ca}W$).



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4.15 The cameo phenotype in Indian blue peafowl is inherited as a Z-linked recessive trait. (a) Blue female crossed with cameo male. (b) Reciprocal cross of cameo female with homozygous blue male.

Description

The illustration “a” shows P generation where blue female with genotype big Z superscript big C small A positive big W is crossed with cameo male with genotype big Z superscript small C small A big Z superscript small C small A. Meiosis occurs and the gametes produced are as follows: Z superscript big C small A positive and big W from the blue female and big Z superscript small C small A from the cameo male. Fertilization occurs and F subscript 1 generation is produced. A Punnett square with 1 row and 2 columns has the following entries.

The row header represents sperm and is as follows: big Z superscript small C small A.

The column headers represent eggs and are as follows: big Z superscript big C small A positive and big W.

Row 1 column 1: big Z superscript big C small A positive big Z superscript small C small A (blue male)

Row 1 column 2: big Z superscript small C small A big W (cameo female). Meiosis occurs and the gametes produced from F subscript 1 generation are as follows: big Z superscript big C small A positive and big Z superscript small C small A from the blue male and big Z superscript small C small A and big W from the cameo female. Fertilization occurs and F subscript 2 generation is obtained. A Punnett square with 2 rows and 2 columns has the following entries:

The row headers represent sperms and are as follows: big Z superscript big C small A positive and big Z superscript small C small A.

The column headers represent eggs and are as follows: big Z superscript small C small A and big W.

Row 1 column 1: big Z superscript big C small A positive big Z superscript small C small A (blue male).

Row 1 column 2: big Z superscript big C small A positive big W (blue female).

Row 2 column 1: big Z superscript small C small A big Z superscript small C small A (cameo male).

Row 2 column 2: big Z superscript small C small A big W (cameo female).

A caption below reads, “Conclusion: one-fourth blue males, one-fourth cameo males, one-fourth blue females, and one-fourth cameo females.”

The illustration “b” shows a cameo female with genotype big Z superscript small C small A crossed with a blue male with genotype big Z superscript big C small A positive. Meiosis occurs and the gametes produced are as follows: big Z superscript small C small A and big W from the cameo female and big Z superscript big C small A positive from the blue male. Fertilization occurs and F subscript 1 generation is obtained. A Punnett square with 1 row and 2 columns has the following entries:

The row header represents sperm and is as follows: big Z superscript big C small A positive.

The column headers represent eggs and are as follows: big Z superscript small C small A and big W.

Row 1 column 1: big Z superscript big C small A positive big Z superscript small C small A (blue male).

Row 1 column 2: big Z superscript big C small A positive big W (blue female).

Meiosis occurs and gametes produced from the F subscript 1 generation are as follows: big Z superscript big C small A positive and big Z superscript small C small A from the blue male and big Z superscript big C small A positive and big W from the blue female. Fertilization occurs and F subscript 2 generation is obtained. A Punnett square with 2 rows and 2 columns has the following entries.

The row headers represent sperms and are as follows: big Z superscript big C small A positive and big Z superscript small C small A.

The column headers represent eggs and are as follows: big Z superscript big C small A positive and big W.

Row 1 column 1: big Z superscript big C small A positive big Z superscript big C small A positive (blue male).

Row 1 column 2: big Z superscript big C small A positive big W (blue female).

Row 2 column 1: big Z superscript big C small A positive big Z superscript small C small A (blue male).

Row 2 column 2: big Z superscript small C small A big W (cameo female).

A caption below reads, “Conclusion: one-half blue males, one-fourth blue females, and one-fourth cameo females.”

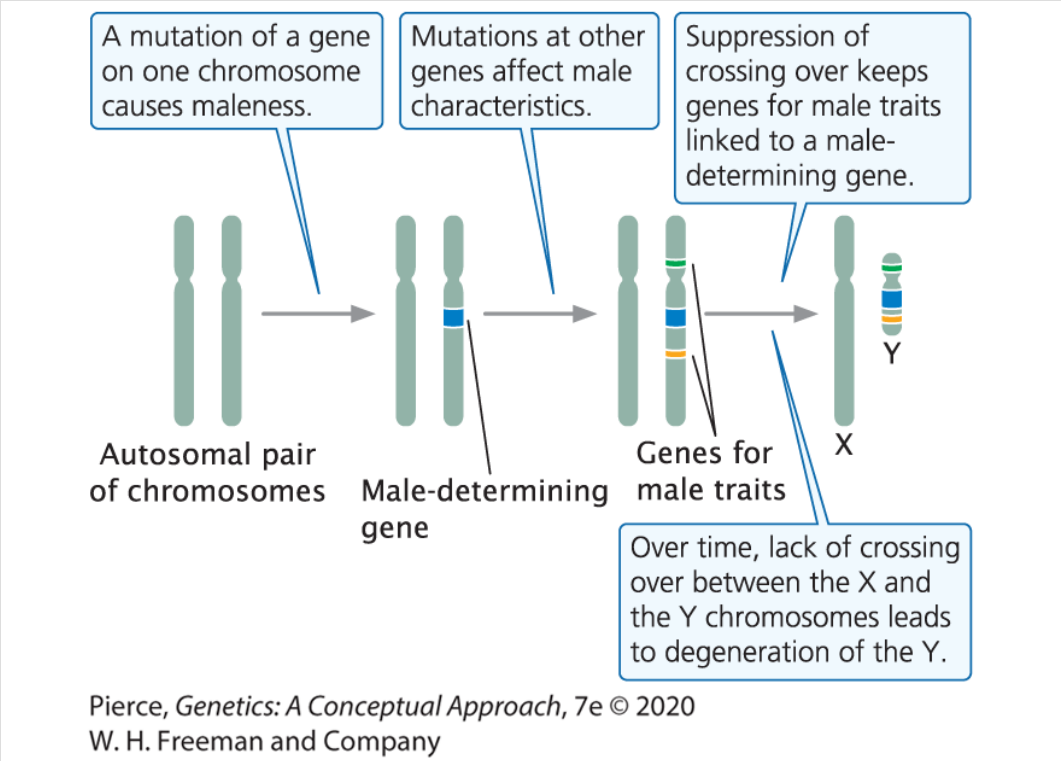
In organisms with ZZ-ZW sex determination, the female always inherits her W chromosome from her mother, and she inherits her Z chromosome, along with any Z-linked alleles, from her father. In this system, the male inherits Z chromosomes, along with any Z-linked alleles, from both his mother and his father. This pattern of inheritance is the reverse of that of X-linked alleles in organisms with XX-XY sex determination. › **TRY** PROBLEM 35

Y-Linked Characteristics

Y-linked traits—also called holandric traits—exhibit a distinct pattern of inheritance. These traits are present only in males, because only males possess a Y chromosome, and are always inherited from the father. Furthermore, all male offspring of a male with a Y-linked trait will display this trait, because every male inherits his Y chromosome from his father. › **TRY** PROBLEM 48

EVOLUTION OF THE Y CHROMOSOME

Research on sex chromosomes has led to the conclusion that the X and Y chromosomes in many organisms evolved from a pair of autosomes. The first step in this evolutionary process took place when one member of a pair of autosomes acquired a gene that determines maleness, such as the *SRY* gene found in humans today (**Figure 4.16**). This step took place in mammals about 250 million years ago. Any individual organism with a copy of the chromosome containing this gene then became male. Additional mutations occurred on the proto-Y chromosome, affecting traits that are beneficial only in males, such as sperm development. The genes that encode these types of traits are advantageous only if they are present in males. Natural selection favors the suppression of crossing over for most of the length of the X and Y chromosomes because this suppression prevents genes that encode male traits from appearing in females. Crossing over can still take place between the two X chromosomes in females, but there is little crossing over between the X and the Y chromosomes, except for the small pseudoautosomal regions in which the X and the Y chromosomes continue to pair in meiosis, as stated earlier.



4.16 Evolution of the Y chromosome.

Description

The illustration shows an autosomal pair of chromosomes where both are of same length and their short arm are shorter than long arm. A mutation of a gene occurs in the second chromosome. A caption reads, “A mutation of a gene on one chromosome causes maleness.” A blue band below centromere of the second chromosome represents male determining gene. Mutations occur at other genes. A caption reads, “Mutations at other genes affect male characteristics.” The second chromosome now has two genes for male traits: a green colored band representing one gene is above the centromere and another yellow colored band representing other gene is below the centromere (also below the male determining gene). A caption reads, “Over time, lack of crossing over between the X and the Y chromosomes leads to degeneration of the Y.” Another caption reads, “Suppression of crossing over keeps genes for male traits linked to male determining gene.” Finally, one X chromosome remains intact but the other chromosome of the autosomal pair with male-determining gene becomes extremely shorter and relatively thicker. This Y chromosome has a male determining gene and two genes for male traits.

For reasons that are beyond the scope of this discussion, the lack of crossing over led to (and continues to lead to) an accumulation of mutations on and a loss of genetic material from the Y chromosome (see Figure 4.16). Over millions of years, the Y chromosome slowly degenerated, losing DNA and genes until it became greatly reduced in size and contained little genetic information. This degeneration produced the Y chromosome found in males today. Indeed, the Y chromosomes of humans and many other organisms are small and contain little genetic information; therefore, few characteristics exhibit Y-linked inheritance. Some researchers have predicted that the human Y chromosome will continue to lose genetic information in the future and will completely disappear from the species in about 10 million years, a disheartening prospect for those of us with a Y chromosome (and perhaps some of those with two Xs). However, new research suggests that decay of the human Y chromosome has come to a halt and that no genes have been lost in the last 6 million years. The genes that remain on the Y chromosome appear to be remarkably stable. Internal recombination within the Y chromosome (described in the next section) may have aided in slowing down or preventing the complete decay of the human Y chromosome.

Among animals, sex chromosomes have independently evolved from autosomes many times. This phenomenon has even been observed in mammals: the sex chromosomes of monotremes (egg-laying mammals such as the duck-billed platypus) evolved from different

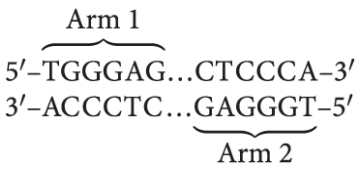
autosomes than did the sex chromosomes of placental and marsupial mammals.

THINK-PAIR-SHARE Question 7

CHARACTERISTICS OF THE HUMAN Y CHROMOSOME

The genetic sequence of most of the human Y chromosome was determined as part of the Human Genome Project (see Chapter 20). This work revealed that about two-thirds of the Y chromosome consists of short DNA sequences that are repeated many times and contain no active genes. The other third consists of just a few genes. Only about 350 genes have been identified on the human Y chromosome, compared with thousands on most chromosomes, and only about half of those identified encode proteins. Some of the protein-encoding genes found on the Y chromosome have homologous genes on the X chromosome. The function of most Y-linked genes is poorly understood; many appear to influence male sexual development and fertility. Others play a role in gene regulation and protein stability. Some are expressed throughout the body, but many are expressed predominantly or exclusively in the testes. Although the Y chromosome has relatively few genes, research with *Drosophila* suggests that it carries genetic elements that affect the expression of numerous genes on autosomes and X chromosomes.

A surprising feature revealed by sequencing is the presence of eight massive palindromic sequences on the Y chromosome. A palindrome is a sentence or word, such as *rotator*, that reads the same backward and forward. A palindromic sequence in DNA is a sequence that reads the same on both strands of the double helix, creating two nearly identical copies stretching out from a central point, such as



Description
The illustration shows both the template and complementary strands. From 5 prime to 3 prime the sequence is as follows: (arm 1 begins) big T big G big G big G big A big G (arm 1 ends) ellipsis big C big T big C big C big C big A. The complementary strand has the following sequence from 3 prime to 5 prime: big A big C big C big C big T big C ellipsis (arm 2 begins) big G big A big G big G big G big T (arm 2 ends).

Thus, a palindromic sequence in DNA appears twice, very much like the two copies of a DNA sequence that are found on two homologous chromosomes. And just as it does with homologous chromosomes, recombination can take place between the two palindromic sequences on the Y chromosome. As already mentioned, the X and the Y chromosomes are not homologous at most of their sequences, and most of the Y chromosome does not undergo crossing over with the X chromosome. This lack of interchromosomal recombination leads to an accumulation of deleterious mutations on the Y

chromosome and the loss of genetic material. Evidence suggests that the two arms of the Y chromosome recombine with each other, which partly compensates for the absence of recombination between the X and the Y chromosomes. This internal recombination may help to maintain some sequences and functions of genes on the Y chromosome and prevent its total degeneration.

Although the palindromic sequences afford opportunities for recombination, which helps prevent the decay of the Y chromosome over evolutionary time, they occasionally have harmful effects. Recent research has revealed that recombination between the palindromes can lead to rearrangements of the Y chromosome that cause anomalies of sexual development. In some cases, recombination between the palindromes leads to deletion of the *SRY* gene, producing an XY female. In other cases, recombination deletes other Y-chromosome genes that take part in sperm production. Sometimes recombination produces a Y chromosome with two centromeres; such a chromosome may break as the centromeres are pulled in opposite directions in mitosis. The broken Y chromosomes may be lost in mitosis, resulting in XO cells and Turner syndrome.

CONCEPTS

Y-linked traits exhibit a distinct pattern of inheritance: they are present only in males, and all male offspring of a male with a Y-linked trait inherit the trait. Palindromic sequences within the Y chromosome can undergo internal recombination, but such recombination may lead to chromosome anomalies.

✔ CONCEPT CHECK 7

What unusual feature of the Y chromosome allows some recombination among the genes found on it?

THE USE OF Y-LINKED GENETIC MARKERS

DNA sequences in the Y chromosome undergo mutation with the passage of time and thus vary among individual males. These mutations create variations in DNA sequence that, like Y-linked traits, are passed from father to son and can therefore be used as genetic markers to study male ancestry. Although the markers themselves do not encode any physical traits, they can be detected with the use of molecular methods. Mutations can readily accumulate in the Y chromosome because so much of it is nonfunctional. Many of these mutations are unique; they arise only once and are passed down through the generations. Individual males possessing the same set of mutations are therefore assumed to be related, and the distribution of these genetic markers on Y chromosomes provides clues about the genetic relationships of present-day people.

Y-linked genetic markers have been used to study the offspring of Thomas Jefferson, principal author of the Declaration of Independence and third president of the United States. In 1802, a political enemy accused Jefferson of fathering a child by his slave

Sally Hemings, but the evidence was circumstantial. Hemings, who worked in the Jefferson household and accompanied Jefferson on a trip to Paris, had five children. Jefferson was accused of fathering the first child, but rumors about the paternity of the other children circulated as well. Descendants of Hemings's children maintained that they were part of the Jefferson line, but some Jefferson descendants refused to recognize their claim.

To resolve this long-standing controversy, geneticists examined markers from the Y chromosomes of male-line descendants of Hemings's first son (Thomas Woodson), her last son (Eston Hemings), and a paternal uncle of Thomas Jefferson with whom Jefferson had Y chromosomes in common (descendants of Jefferson's uncle were used because Jefferson himself had no verified male descendants). Geneticists determined that Jefferson possessed a rare and distinctive set of genetic markers on his Y chromosome. The same markers were also found on the Y chromosomes of the male-line descendants of Eston Hemings. The probability of such a match arising by chance is less than 1%. The markers were not found on the Y chromosomes of the descendants of Thomas Woodson. Together with the circumstantial historical evidence, these matching markers suggest that Jefferson (or a male-line relative of Jefferson) was the father of Eston Hemings but not Thomas Woodson.

Y-chromosome sequences have also been used extensively to examine past patterns of male migration and the genetic relationships among different human populations. Female lineages

can be traced through sequences on mitochondrial DNA, which are inherited from the mother (see Chapter 11).

CONNECTING CONCEPTS

Recognizing Sex-Linked Inheritance

What features should we look for to identify a trait as sex linked? A common misconception is that any genetic characteristic in which the phenotypes of males and females differ must be sex linked. In fact, the expression of many *autosomal* characteristics differs between males and females. The genes that encode these characteristics are the same in both sexes, but their expression is influenced by sex hormones. The different sex hormones of males and females cause the same genes to generate different phenotypes in males and females.

Another misconception is that any characteristic that is found more frequently in one sex than in the other is sex linked. A number of autosomal traits are expressed more commonly in one sex than in the other. These traits are said to be sex-influenced. Some autosomal traits are expressed in only one sex; these traits are said to be sex-limited. Both sex-influenced and sex-limited characteristics will be considered in more detail in Chapter 5.

Several features of sex-linked characteristics make them easy to recognize. Y-linked traits are found only in males, but observing a trait only in males does not guarantee that it is Y linked because some autosomal characteristics are expressed only in males. Y-linked traits are unique, however, in that *all* the male offspring of an affected male express the father's phenotype. Furthermore, a Y-linked trait can be inherited only from the father's side of the family. Thus, a Y-linked trait can be inherited only from the paternal grandfather (the father's father), never from the maternal grandfather (the mother's father).

X-linked characteristics also exhibit a distinctive pattern of inheritance. X linkage is a possible explanation when reciprocal crosses give different results. If a characteristic is X linked, a cross between an affected male and an unaffected female will not give the same results as a cross between an affected female and an unaffected male. For almost all autosomal characteristics, reciprocal crosses give the same result. We should not conclude, however, that when the reciprocal crosses give different results, the characteristic is necessarily X linked. Other sex-associated forms of inheritance, considered in Chapter 5, also produce different results in reciprocal crosses. The key to recognizing X-linked inheritance is to remember that a

male always inherits his X chromosome from his mother, not from his father. Thus, an X-linked characteristic is not passed directly from father to son; if a male clearly inherits a trait from his father—and his mother is not heterozygous—it cannot be X linked.

4.3 Dosage Compensation Equalizes the Amount of Protein Produced by X-Linked and Autosomal Genes in Some Animals

In species with XX-XY sex determination, differences between males and females in their number of X chromosomes present a special problem in development. In females, there are two copies of the X chromosome and two copies of each autosome, so genes on the X chromosomes and on autosomes are "in balance." In males, however, there is only a single X chromosome, while there are two copies of every autosome. Because the amount of a protein produced is often a function of the number of gene copies encoding that protein, males are likely to produce smaller amounts of a protein encoded by X-linked genes than of a protein encoded by autosomal genes. This difference can be detrimental because protein concentration often plays a critical role in development.

Some animals have overcome this problem by evolving mechanisms to equalize the amounts of protein produced by the single X and by two autosomes in the heterogametic sex. These mechanisms are referred to as **dosage compensation**. In fruit flies, dosage compensation is achieved by a doubling of the activity of the genes on the X chromosome of males, but not that of females. In placental mammals, the expression of dosage-sensitive genes on

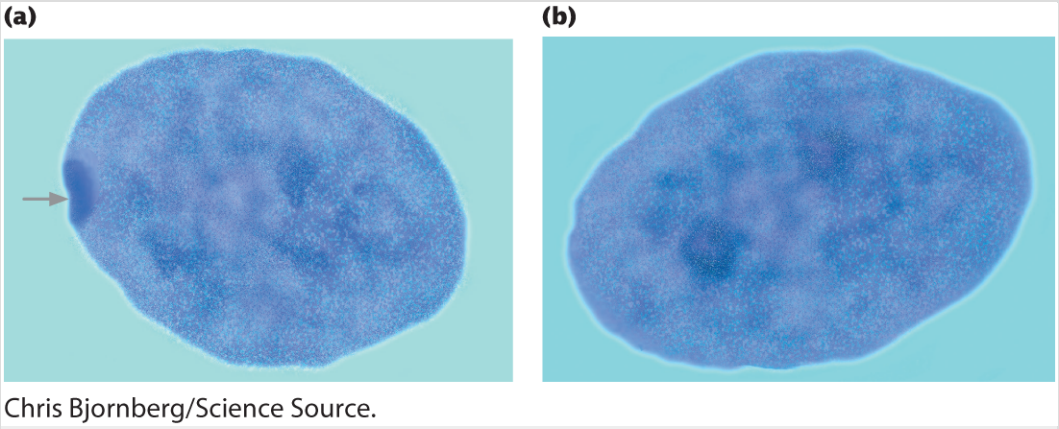
the X chromosomes of both males and females has increased, coupled with inactivation of one of the X chromosomes in females, so that expression of X-linked and autosomal genes is balanced in both males and females.

For unknown reasons, the presence of sex chromosomes does not always produce problems of gene dosage, and dosage compensation of X-linked genes is not universal. A number of animals do not exhibit obvious mechanisms of dosage compensation; these animals include butterflies and moths, birds, some fishes, and even the duck-billed platypus. As we see in the next section, even in placental mammals, a number of genes escape dosage compensation.

The Lyon Hypothesis

In 1949, Murray Barr observed condensed, darkly staining bodies in the nuclei of cells from female cats (**Figure 4.17**); these structures became known as **Barr bodies**. Mary Lyon proposed, in 1961, that the Barr body was an inactive X chromosome; her hypothesis (now generally accepted for placental mammals) has become known as the **Lyon hypothesis**. She suggested that within each female cell, one of the two X chromosomes is inactivated. Which X chromosome is inactivated is random; if a cell contains more than two X chromosomes, all but one of them are inactivated. The

number of Barr bodies present in human cells with different complements of sex chromosomes is shown in Table 4.3. Lyon's hypothesis led to important insights into the process of development, the expression of X-linked traits, and X-linked genetic diseases.



Chris Bjornberg/Science Source.

4.17 A Barr body is an inactivated X chromosome. (a) Female cell with a Barr body (indicated by arrow). (b) Male cell without a Barr body.

TABLE 4.3 Number of Barr bodies in human cells with different complements of sex chromosomes

Sex Chromosomes	Syndrome	Number of Barr Bodies
XX	None	1
XY	None	0
XO	Turner	0

XXY	Klinefelter	1
XXYY	Klinefelter	1
XXXY	Klinefelter	2
XXXXY	Klinefelter	3
XXX	Triple-X	2
XXXX	Poly-X female	3
XXXXX	Poly-X female	4

One of the outstanding geneticists of the twentieth century, Mary Lyon (**Figure 4.18**) conducted significant research in mouse genetics, including important work on mutagenesis, chromosome inversions, and the *t* complex, a genetic element on mouse chromosome 17 that causes some chromosomes to be preferentially transmitted during meiosis. Lyon also developed many techniques that are used today in mouse genetics, helping to make the mouse an important model genetic organism.

© Miron Latyszewski, Courtesy MRC Harwell/Mary Lyon.

4.18 Mary Lyon.

As a result of X inactivation, female placental mammals are functionally hemizygous at the cellular level for X-linked genes. In females that are heterozygous at an X-linked locus, approximately 50% of the cells express one allele and 50% express the other allele; thus, in heterozygous females, proteins encoded by both alleles are produced, but not within the same cell. This functional hemizygosity means that the cells in an individual female are not identical with respect to the expression of the genes on the X

chromosome; females are mosaics for the expression of X-linked genes.

Random X inactivation takes place early in development—in humans, it occurs within the first few weeks of development. After an X chromosome has become inactivated in a cell, it remains inactive in that cell and in all somatic cells that descend from that cell. Thus, neighboring cells tend to have the same X chromosome inactivated, producing a patchy pattern (mosaic) for the expression of an X-linked characteristic in heterozygous females.

This patchy distribution of gene expression can be seen in tortoiseshell and calico cats (**Figure 4.19**). Although many genes contribute to coat color and pattern in domestic cats, a single X-linked locus determines the presence of orange color. There are two possible alleles at this locus: X^+, which produces non-orange (usually black) fur, and X^o, which produces orange fur. Males are hemizygous and thus may be black (X^+Y) or orange (X^oY) but not black and orange. (Rare tortoiseshell males can arise from the presence of two X chromosomes, X^+X^oY.) Females may be black (X^+X^+), orange (X^oX^o), or tortoiseshell (X^+X^o), with the tortoiseshell pattern arising from a patchy mixture of black and orange fur. Each orange patch is a clone of cells derived from an original cell in which the black allele was inactivated, and each black patch is a clone of cells derived from an original cell in which the orange allele was inactivated.

Robert Adrian Hillman/Shutterstock.

4.19 The patchy distribution of color on tortoiseshell cats results from the random inactivation of one X chromosome in females.

The Lyon hypothesis suggests that the presence of variable numbers of X chromosomes should not affect the phenotype in mammals because any X chromosomes in excess of one should be inactivated. However, people with Turner syndrome (XO) differ from XX females, and those with Klinefelter syndrome (XXY) differ from XY males. How do the phenotypes of these conditions arise in the face of dosage compensation?

The phenotypes associated with these conditions probably arise because some X-linked genes escape inactivation. Indeed, the nature of X inactivation is more complex than originally envisioned. Studies of individual genes now reveal that only about 75% of X-linked human genes are permanently inactivated. About 15% completely escape X inactivation, meaning that these genes produce twice as much protein in females as they do in males. The

remaining 10% are inactivated in some females but not in others. The reason for this variation among females is not known. Furthermore, recent research indicates that X inactivation does not actually equalize dosage of many X-linked and autosomal genes in humans and mice. › **TRY** PROBLEM 47

Mechanism of Random X Inactivation

Random inactivation of X chromosomes requires two steps. In the first step, the cell somehow assesses, or counts, how many X chromosomes are present. In the second step, one X chromosome is selected to become the active X chromosome, and all others are inactivated.

Although some details of X-chromosome inactivation remain unknown, several genes and sequences that participate in the process have been identified. Foremost among them is a gene called *Xist* (for *X-i*nactive *s*pecific *t*ranscript). On the X chromosomes destined to be inactivated, the *Xist* gene is active, producing a 17,000-nucleotide-long RNA molecule that coats the X chromosome and inactivates the genes on it by recruiting protein complexes that alter chromatin structure. On the X chromosome destined to be active, other genes repress the activity of *Xist* so that the *Xist* RNA never coats the X chromosome, and genes on this chromosome

remain active. The mechanism of X inactivation is discussed in more detail in Chapter 21.

CONCEPTS

In placental mammals, all but one X chromosome are inactivated in each cell; which of the X chromosomes is inactivated is random and varies from cell to cell.

✔ CONCEPT CHECK 8

How many Barr bodies does a male with XXXYY chromosomes have in each of his cells?

Chapter 4 Review

CHAPTER SUMMARY

Section 4.1 Learning Objective: Be able to outline the different mechanisms by which sex may be determined.

- Sexual reproduction is the production of offspring that are genetically distinct from their parents. Most organisms have two sexual phenotypes—males and females. Males produce small gametes; females produce large gametes.
- The mechanism by which sex is specified is termed sex determination. Sex may be determined by differences in specific chromosomes, genotypes, or environment.
- The sex chromosomes of males and females differ in number and appearance. The homogametic sex produces gametes that are all identical with regard to sex chromosomes; the heterogametic sex produces gametes that differ in their sex-chromosome composition.
- In the XX-XO system of sex determination, females possess two X chromosomes, whereas males possess a single X chromosome. In the XX-XY system, females possess two X chromosomes, whereas males possess a single X chromosome and a single Y chromosome. In the ZZ-ZW system, males possess two Z chromosomes, whereas females possess a Z chromosome and a W chromosome.

- Some organisms have genic sex determination, in which genotypes at one or more loci determine the sex of an individual organism. Still others have environmental sex determination.
- In *Drosophila melanogaster*, sex can be predicted by the X : A ratio but is primarily determined by genes on the X chromosome.
- In humans, sex is ultimately determined by the presence or absence of the *SRY* gene located on the Y chromosome.

Section 4.2 Learning Objective: Be able to predict the types and proportions of progeny produced in crosses involving traits determined by genes on sex chromosomes.

- Sex-linked characteristics are determined by genes on the sex chromosomes. X-linked characteristics are encoded by genes on the X chromosome, and Y-linked characteristics are encoded by genes on the Y chromosome.
- A female inherits X-linked alleles from both parents; a male inherits X-linked alleles from his female parent only.
- Y-linked characteristics are found only in males and are passed from a father to all his sons.
- The sex chromosomes evolved from autosomes. Crossing over between the X and the Y chromosomes has been suppressed, but palindromic sequences within the Y chromosome allow for internal recombination on the Y chromosome. This internal recombination sometimes leads to chromosome rearrangements that can adversely affect sexual development.

Section 4.3 Learning Objective: Be able to explain how dosage compensation equalizes the amount of protein produced by X-linked and autosomal genes.

- In placental mammals, one of the two X chromosomes in females is normally inactivated. Which X chromosome is inactivated is random and varies from cell to cell. Some X-linked genes escape X inactivation, and other X-linked genes may be inactivated in some females but not in others. X inactivation is controlled by the *Xist* gene.

WORKED PROBLEMS

Problem 1

A fruit fly has XXXYY sex chromosomes; all the autosomal chromosomes are normal. What sexual phenotype does this fly have?

» Solution Strategy

What information is required in your answer to the problem?

The sexual phenotype of a fly with sex chromosomes XXXYY.

What information is provided to solve the problem?

- The fly has sex chromosomes XXXYY.

- All autosomal chromosomes are normal.

For help with this problem, review:

Sex Determination in *Drosophila melanogaster* in Section 4.1.

» Solution Steps

Sex in fruit flies is predicted by the X : A ratio—the ratio of the number of X chromosomes to the number of haploid autosomal sets. An X : A ratio of 1.0 produces a female fly; an X : A ratio of 0.5 produces a male. If the X : A ratio is greater than 1.0, the fly is a metafemale; if it is less than 0.5, the fly is a metamale; if the X : A ratio is between 1.0 and 0.5, the fly is intersex.

This fly has three X chromosomes and normal autosomes, so the X : A ratio in this case is $^{3}/_{2}$, or 1.5. Thus, this fly is a metafemale.

> **Recall:** *Drosophila melanogaster* normally has two sets of autosomes.

Problem 2

In *Drosophila melanogaster*, forked bristles are caused by an allele (X^{f}) that is X linked and recessive to an allele for normal bristles (X^{+}). Brown eyes are caused by an allele (b) that is autosomal and recessive to an allele for red eyes (b^{+}). A female fly that is

homozygous for normal bristles and red eyes mates with a male fly that has forked bristles and brown eyes. The F_1 are intercrossed to produce the F_2. What will be the phenotypes and proportions of the F_2 flies from this cross?

» Solution Strategy

What information is required in your answer to the problem?

Phenotypes and proportions of the F_2 flies.

What information is provided to solve the problem?

- Forked bristles are X-linked recessive.
- Brown eyes are autosomal recessive.
- Phenotypes of the parents of the cross.
- The F_1 are intercrossed to produce the F_2.

For help with this problem, review:

X-linked Color Blindness in Humans in Section 4.2.

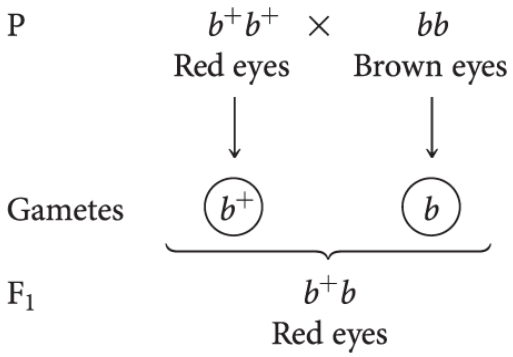
Section 3.3 in Chapter 3.

» Solution Steps

This problem is best worked by breaking the cross down into two separate crosses, one for the X-linked genes that determine the type

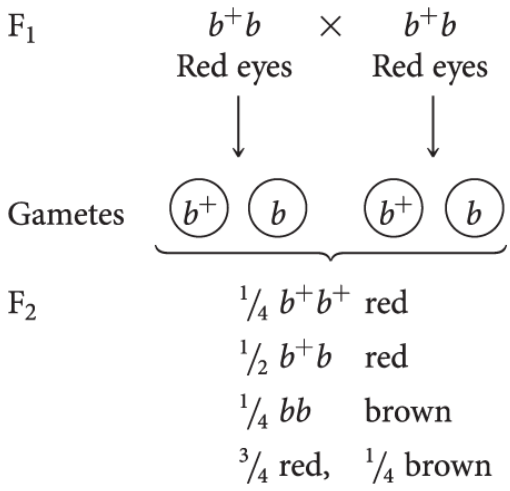
of bristles and one for the autosomal genes that determine eye color.

Let's begin with the autosomal characteristics. A female fly that is homozygous for red eyes (b^+b^+) is crossed with a male with brown eyes. Because brown eyes are recessive, the male fly must be homozygous for the brown-eye allele (bb). All the offspring of this cross will be heterozygous (b^+b) and will have red eyes:



Description
Genotype of the parent fly (P generation) for red eyes is as follows: small B superscript positive small B superscript positive. Genotype of the parent fly for brown eyes is as follows: small B small B. Genotypes of the gametes produced are as follows: small B superscript positive and small B, respectively. The F subscript 1 progeny produced from the cross has red eyes with small B superscript positive small B.

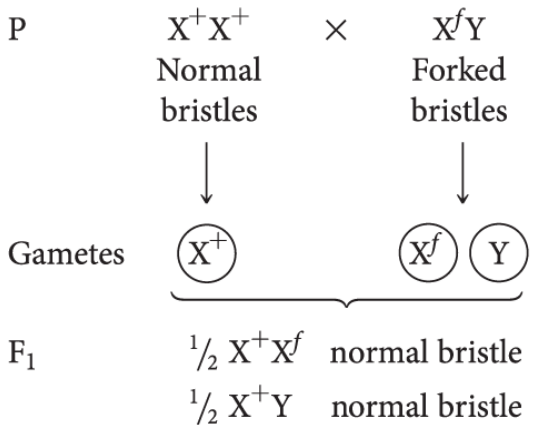
The F_1 are then intercrossed to produce the F_2. Whenever two individual organisms heterozygous for an autosomal recessive characteristic are crossed, $^3/_4$ of the offspring will have the dominant trait, and $^1/_4$ will have the recessive trait; thus, $^3/_4$ of the F_2 flies will have red eyes, and $^1/_4$ will have brown eyes:



Description

The illustration shows F subscript 1 generation in which two red-eyed flies with genotype small B superscript positive small B are intercrossed. The gametes obtained from each of them are as follows: small B superscript positive and small B. F subscript 2 generation is shown below the gametes and their genotypes are as follows: one-fourth small B superscript positive small B superscript positive (red); one-half small B superscript positive small B (red); one-fourth small B small B (brown). A text below reads three-fourth red and one-fourth brown.

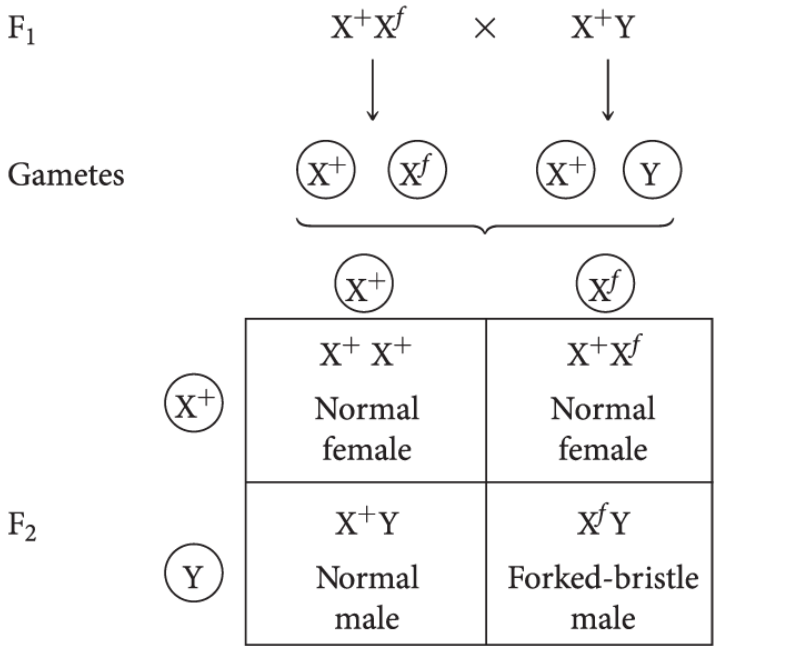
Next, we work out the results for the X-linked characteristic. A female that is homozygous for normal bristles (X^+X^+) is crossed with a male that has forked bristles (X^fY). Thefemale F_1 from this cross are heterozygous (X^+X^f), receiving an X chromosome with a normal-bristle allele (X^+) from their mother and an X chromosome with a forked-bristle allele (X^f) from their father. The male F_1 are hemizygous (X^+Y), receiving an X chromosome with a normal-bristle allele (X^+) from their mother and a Y chromosome from their father:



Description
Genotype of the parent (P generation) with normal bristles is as follows: big X superscript positive big X superscript positive. Genotype of the parent with forked bristles is as follows: big X superscript small F big Y. Genotypes of the gametes produced are as follows: big X superscript positive and big X superscript positive and big Y, respectively. Half of the progenies produced in the F subscript 1 generation have big X superscript positive big X superscript small F (normal bristles) and the other half have genotype big X superscript positive big Y (normal bristles).

Recall: Females have two X-linked alleles, but males have only a single X-linked allele.

When these F_1 are intercrossed, $^1/_2$ of the F_1 will be normal-bristle females, $^1/_4$ will be normal-bristle males, and $^1/_4$ will be forked-bristle males:



½ normal female, ¼ normal male, ¼ forked-bristle male

Description

The illustration shows female with genotype, big X superscript positive big X superscript small F, is crossed with male with big X superscript positive big Y. Genotypes of the gametes produced are as follows: big X superscript positive, big X superscript small F from big X superscript positive big X superscript small F and big X superscript positive and big Y from big X superscript positive big Y. F subscript 2 generation is obtained. A punnett square having 2 rows and 2 columns shows the following entries:

Row headers are as follows: big X superscript positive and big Y.

Column headers are as follows: big X superscript positive and big X superscript small F.

Row 1 column 1: big X superscript positive big X superscript positive.

Row 1 column 2: big X superscript positive big X superscript small F.

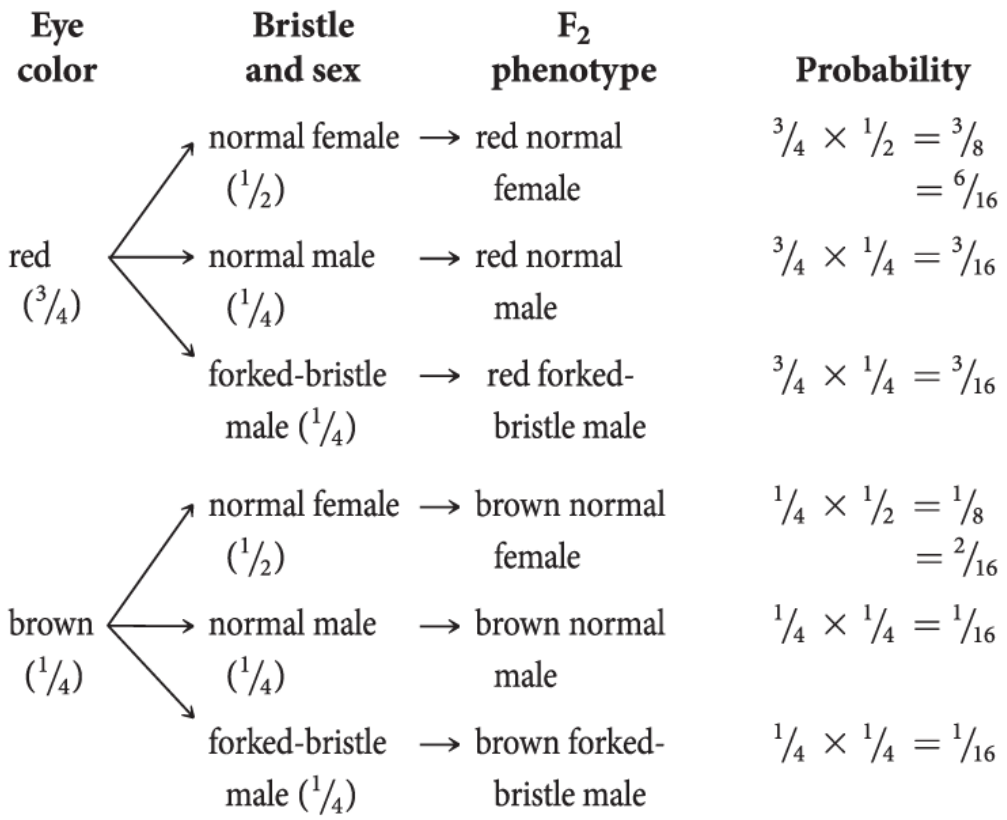
Row 2 column 1: big X superscript positive big Y.

Row 2 column 2: big X superscript small F big Y.

Text at the bottom reads one-half normal female, one-half normal male, and one-fourth forked-bristle male.

To obtain the phenotypic ratio in the F_2, we now combine these two crosses by using the multiplication rule and a branch diagram:

Recall: The multiplication rule states that the probability of two independent events occurring together is the multiplication of their independent probabilities.



Description
First tier of the branch diagram on the left shows eye color as follows: three-fourth red and one-fourth brown. Second tier of the branch diagram to its right shows bristle and sex as follows: red normal female one-half, red normal male one-fourth, and red forked-bristle male one-fourth, brown normal female one-half, brown normal male one-fourth, and brown forked-bristle male one-fourth. Third tier of the branch diagram on the right shows F subscript 2 phenotype and the fourth tier on the extreme right shows probability for each of the phenotype of third tier respectively as follows: red normal female; three-fourth multiplied by one-half equals three-eighth equals six over sixteen, red normal male; three-

fourth multiplied by one-fourth equals three over sixteen, red forked-bristle male; three-fourth multiplied by one-fourth equals three over sixteen, brown normal female; one-fourth multiplied by one-half equals one-eighth equals two over sixteen, brown normal male; one-fourth multiplied by one-fourth equals one over sixteen, and brown forked-bristle male; one-fourth multiplied by one-fourth equals one over sixteen. The first three entries from second, third, and fourth tier correspond to red eye color and the last three entries from second, third, and fourth tier correspond to brown eye color.

Hint: The branch diagram is a convenient way of keeping up with all the different combinations of traits.

IMPORTANT TERMS

sex
sex determination
gender
hermaphroditism
monoecious
dioecious
sex chromosome
autosome
heterogametic sex
homogametic sex
pseudoautosomal region
genic sex determination
sequential hermaphroditism
Turner syndrome
Klinefelter syndrome

triple-X syndrome
sex-determining region Y (*SRY*) gene
sex-linked characteristic
X-linked characteristic
Y-linked characteristic
hemizygous
nondisjunction
dosage compensation
Barr body
Lyon hypothesis

ANSWERS TO CONCEPT CHECKS

1. Meiosis
2. b
3. In chromosomal sex determination, males and females have chromosomes that are distinguishable. In genic sex determination, sex is determined by genes, but the chromosomes of males and females are indistinguishable. In environmental sex determination, sex is determined fully or in part by environmental effects.
4. a
5. c
6. All of their male offspring will have hemophilia, and none of their female offspring will have hemophilia, so the overall probability of hemophilia in their offspring is $^1/_2$.

7. Eight large palindromes allow crossing over within the Y chromosome.
8. Two Barr bodies.

Chapter 4 Assessment

COMPREHENSION QUESTIONS

Section 4.1

1. What is considered to be the fundamental difference between males and females of most organisms?

2. How do monoecious organisms differ from dioecious organisms?

3. Describe the XX-XO system of sex determination. In this system, which is the heterogametic sex, and which is the homogametic sex?

4. *How does sex determination in the XX-XY system differ from sex determination in the ZZ-ZW system?

5. What is the pseudoautosomal region? How does the inheritance of traits encoded by genes in this region differ from the inheritance of other Y-linked characteristics?

6. What is meant by genic sex determination?

7. How does sex determination in *Drosophila* differ from sex determination in humans?

8. Give the typical sex-chromosome complement found in the cells of people with Turner syndrome, with Klinefelter syndrome, and with androgen-insensitivity syndrome. What is the sex-chromosome complement of triple-X females?

Section 4.2

9. What characteristics are exhibited by an X-linked trait?

10. Explain how Bridges's study of nondisjunction in *Drosophila* helped prove the chromosome theory of inheritance.

11. What characteristics are exhibited by a Y-linked trait?

Section 4.3

12. Explain why tortoiseshell cats are almost always female and why they have a patchy distribution of orange and black fur.

13. What is a Barr body? How is it related to the Lyon hypothesis?

APPLICATION QUESTIONS AND PROBLEMS

Introduction

14. As discussed in the introduction to this chapter, sex in bearded dragons is determined by both sex chromosomes and temperature. What, if any, would be the effect of unusually cool

temperatures during the breeding season on the sex ratio of bearded dragons? What would be the effect of unusually warm temperatures on the sex ratio?

Section 4.1

15. *What is the sexual phenotype of fruit flies having the following chromosomes?

Sex chromosomes	Autosomal chromosomes
a. XX	all normal
b. XY	all normal
c. XO	all normal
d. XXY	all normal
e. XYY	all normal
f. XXYY	all normal
g. XXX	all normal
h. XX	four haploid sets
i. XXX	four haploid sets
j. XXX	three haploid sets

k. X	three haploid sets
l. XY	three haploid sets
m. XX	three haploid sets

16. CONNECT If nondisjunction of the sex chromosomes takes place in meiosis I (see Chapter 2) in the male in **Figure 4.5**, what sexual phenotypes and proportions of offspring will be produced?

17. *For each of the following chromosome complements, what is the phenotypic sex of a person who has

    a. XY with the *SRY* gene deleted?

    b. XX with a copy of the *SRY* gene on an autosomal chromosome?

    c. XO with a copy of the *SRY* gene on an autosomal chromosome?

    d. XXY with the *SRY* gene deleted?

    e. XXYY with one copy of the *SRY* gene deleted?

18. A normal female *Drosophila* produces abnormal eggs that contain all of her chromosomes (a complete diploid set). She mates with a normal male *Drosophila* that produces normal sperm. What will the sex of the progeny from this cross be?

19. In certain salamanders, the sex of a genetic female can be altered, changing her into a functional male; these salamanders are called sex-reversed males. When a sex-reversed male is mated with a normal female, approximately $^2/_3$ of the offspring are female and $^1/_3$ are male. How is sex determined in these salamanders? Explain the results of this cross.

20. In some mites, males pass genes to their grandsons, but they never pass genes to their sons. Explain.

21. *In organisms with the ZZ-ZW sex-determining system, from which of the following possibilities can a female inherit her Z chromosome?

	Yes	No
Her mother's mother	_____	_____
Her mother's father	_____	_____
Her father's mother	_____	_____
Her father's father	_____	_____

Section 4.2

22. *When Bridges crossed white-eyed females with red-eyed males, he obtained a few red-eyed males and white-eyed females (see **Figure 4.13**). What types of offspring would be produced if these red-eyed males and white-eyed females were crossed with each other?

23. *Joe has classic hemophilia, an X-linked recessive disease. Could Joe have inherited the gene for this disease from the following people?

	Yes	No
a. His mother's mother	_____	_____
b. His mother's father	_____	_____
c. His father's mother	_____	_____
d. His father's father	_____	_____

24. *In *Drosophila*, yellow body color is due to an X-linked gene that is recessive to the gene for gray body color.

Courtesy Dr. Masa-Toshi Yamamoto, Drosophila Genetic Resource Center, Kyoto Institute of Technology.

a. A homozygous gray female is crossed with a yellow male. The F_1 are intercrossed to produce the F_2. Give the genotypes and phenotypes, along with the expected proportions, of the F_1 and F_2 progeny.

b. A yellow female is crossed with a gray male. The F_1 are intercrossed to produce the F_2. Give the genotypes and phenotypes, along with the expected proportions, of the F_1 and F_2 progeny.

c. A yellow female is crossed with a gray male. The F_1 females are backcrossed with gray males. Give the genotypes and phenotypes, along with the expected proportions, of the F_2 progeny.

d. If the F_2 flies in part *b* mate randomly, what are the expected phenotypes and proportions of flies in the F_3?

25. Coat color in cats is determined by genes at several different loci. At one locus on the X chromosome, one allele (X^+) encodes black fur; another allele (X^o) encodes orange fur. Females can be black

(X^+X^+), orange (X^oX^o), or a mixture of orange and black called tortoiseshell (X^+X^o). Males are either black (X^+Y) or orange (X^oY). Bill has a female tortoiseshell cat named Patches. One night Patches escapes from Bill's house, spends the night out, and mates with a stray male. Patches later gives birth to the following kittens: one orange male, one black male, two tortoiseshell females, and one orange female. Give the genotypes of Patches, her kittens, and the stray male with which Patches mated.

26. *Red–green color blindness in humans is due to an X-linked recessive gene. Both John and Cathy have normal color vision. After 10 years of marriage to John, Cathy gave birth to a color-blind daughter. John filed for divorce, claiming that he is not the father of the child. Is John justified in his claim of nonpaternity? Explain why. If Cathy had given birth to a color-blind son, would John be justified in claiming nonpaternity?

27. CONNECT Maria has red–green color blindness; Juan has normal color vision. Together, Maria and Juan have two children, Maggi and Daniel. Draw the sex chromosomes of Maggi and Daniel at the following stages, and label on these chromosomes the alleles for color blindness and normal color vision (see Chapter 2 for stages of meiosis). Assume that no crossing over has occurred.

    a. Metaphase of mitosis

    b. Metaphase I of meiosis

    c. Metaphase II of meiosis

28. Red–green color blindness in humans is due to an X-linked recessive gene. A woman whose father is color blind possesses one eye with normal color vision and one eye with color blindness.

    a. Propose an explanation for this woman's vision pattern. Assume that no new mutations have spontaneously arisen.

    b. Would it be possible for a man to have one eye with normal color vision and one eye with color blindness?

29. In 1875, Charles Darwin, author of *On the Origin of Species*, wrote of a peculiar family from Sind, a province in northwest India (C. Darwin. *The Variation of Animals and Plants under Domestication*, 2nd ed. 1875. London: John Murray, London. p. 319):

    > … in which ten men, in the course of four generations, were furnished in both jaws taken together, with only four small and weak incisor teeth and with eight posterior molars. The men thus affected have little hair on the body, and become bald early in life. They also suffer much during hot weather from excessive dryness of the skin. It is remarkable that no instance has occurred of a daughter being thus affected …. Though daughters in the above family are never affected, they transmit the tendency to their sons; and no case has occurred of a son transmitting it to his sons.

    The men in this family had a condition that is now known as hypohidrotic ectodermal dysplasia. Darwin's account is an accurate description of the symptoms and inheritance of this disorder. (a) Based on Darwin's description, what is the most likely mode of inheritance for hypohidrotic ectodermal dysplasia? What evidence in Darwin's description supports your conclusion? (See also Problem 47 and Think-Pair-Share Question 8.)

30. *Bob has XXY chromosomes (Klinefelter syndrome) and is color blind. His mother and father have normal color vision, but his maternal grandfather is color blind. Assume that Bob's chromosome abnormality arose from nondisjunction in meiosis. In which parent and in which meiotic division did nondisjunction take place? Assume no crossing over has taken place. Explain your answer.

31. Xg is an antigen found on red blood cells. This antigen is caused by an X-linked allele (X^a) that is dominant over an allele for the absence of the antigen (X^-). The inheritance of these X-linked alleles was studied in children with chromosome abnormalities to determine where nondisjunction of the sex chromosomes took place. For each type of mating in parts *a* through *d*, indicate whether nondisjunction took place in the mother or in the father and, if possible, whether it took place in meiosis I or meiosis II (assume no crossing over).

    a. $X^aY \times X^-X^- \rightarrow X^a$ (Turner syndrome)

    b. $X^aY \times X^aX^- \rightarrow X^-$ (Turner syndrome)

    c. $X^aY \times X^-X^- \rightarrow X^aX^-Y$ (Klinefelter syndrome)

    d. $X^aY \times X^aX^- \rightarrow X^-X^-Y$ (Klinefelter syndrome)

32. The Talmud, an ancient book of Jewish civil and religious laws, states that if a woman bears two sons who die of bleeding after circumcision (removal of the foreskin from the penis), any additional sons that she has should not be circumcised. (The bleeding is most likely due to the X-linked disorder hemophilia.)

Furthermore, the Talmud states that the sons of her sisters must not be circumcised, whereas the sons of her brothers should be. Is this religious law consistent with sound genetic principles? Explain your answer.

33.

Craniofrontonasal syndrome (CFNS) is a birth defect in which premature fusion of the cranial sutures leads to abnormal head shape, widely spaced eyes, nasal clefts, and various other skeletal abnormalities. George Feldman and his colleagues looked at several families in which offspring had CFNS and recorded the results shown in the following table (G. J. Feldman. 1997. *Human Molecular Genetics* 6:1937–1941).

			Offspring			
Family number	**Parents**		**Normal**		**CFNS**	
	Father	**Mother**	**Male**	**Female**	**Male**	**Female**
1	normal	CFNS	1	0	2	1
5	normal	CFNS	0	2	1	2
6	normal	CFNS	0	0	1	2
8	normal	CFNS	1	1	1	0
10a	CFNS	normal	3	0	0	2
10b	normal	CFNS	1	1	2	0
12	CFNS	normal	0	0	0	1
13a	normal	CFNS	0	1	2	1
13b	CFNS	normal	0	0	0	2
7b	CFNS	normal	0	0	0	2

a. On the basis of these results, what is the most likely mode of inheritance for CFNS?

b. Give the most likely genotypes of the parents in family 1 and in family 10a.

34. Miniature wings (X^m) *in Drosophila* result from an X-linked allele that is recessive to the allele for long wings (X^+). Give the genotypes of the parents in each of the following crosses.

Male parent	Female parent	Male offspring	Female offspring
a. long	Long	231 long, 250 miniature	560 long
b. miniature	Long	610 long	632 long
c. miniature	Long	410 long, 417 miniature	412 long, 415 miniature
d. long	Miniature	753 miniature	761 long
e. long	Long	625 long	630 long

35. *In chickens, congenital baldness is due to a Z-linked recessive gene. A bald rooster is mated with a normal hen. The F_1 from this cross are interbred to produce the F_2. Give the genotypes and

phenotypes, along with their expected proportions, among the F_1 and F_2 progeny.

36. If the blue F_1 females in **Figure 4.15b** are backcrossed to the blue males in the P generation, what phenotypes and proportions of offspring will be produced?

37. Red–green color blindness is an X-linked recessive trait in humans. Polydactyly (extra fingers and toes) is an autosomal dominant trait. Martha has normal fingers and toes and normal color vision. Her mother is normal in all respects, but her father is color blind and polydactylous. Bill is color blind and polydactylous. His mother has normal color vision and normal fingers and toes. If Bill and Martha mate, what phenotypes and proportions of children can they produce?

38. A *Drosophila* mutation called *singed* (*s*) causes the bristles to be bent and misshapen. A mutation called *purple* (*p*) causes the fly's eyes to be purple in color instead of the normal red. Flies homozygous for *singed* and *purple* were crossed with flies that were homozygous for normal bristles and red eyes. The F_1 were intercrossed to produce the F_2, and the following results were obtained.

Cross 1	
P	male, singed bristles, purple eyes × female, normal bristles, red eyes
F_1	420 female, normal bristles, red eyes

	426 male, normal bristles, red eyes
F_2	337 female, normal bristles, red eyes
	113 female, normal bristles, purple eyes
	168 male, normal bristles, red eyes
	170 male, singed bristles, red eyes
	56 male, normal bristles, purple eyes
	58 male, singed bristles, purple eyes

Cross 2	
P	female, singed bristles, purple eyes × male, normal bristles, red eyes
F_1	504 female, normal bristles, red eyes
	498 male, singed bristles, red eyes
F_2	227 female, normal bristles, red eyes
	223 female, singed bristles, red eyes
	225 male, normal bristles, red eyes
	225 male, singed bristles, red eyes

78 female, normal bristles, purple eyes	
76 female, singed bristles, purple eyes	
74 male, normal bristles, purple eyes	
72 male, singed bristles, purple eyes	

a. What are the modes of inheritance of *singed* and *purple?* Explain your reasoning.

b. Give genotypes for the parents and offspring in the P, F_1, and F_2 generations of cross 1 and cross 2.

39. The following two genotypes are crossed: *Aa Bb Cc* $X^+X^r \times$ *Aa BB cc* X^+Y, where *a, b,* and *c* represent alleles of autosomal genes, and X^+ and X^r represent X-linked alleles in an organism with XX-XY sex determination. What is the probability of obtaining genotype *aa Bb Cc* X^+X^+ in the progeny? See Chapter 3 for a review of autosomal inheritance.

40. *Miniature wings in *Drosophila* result from an X-linked allele (X^m) that is recessive to the allele for long wings (X^+). Sepia eyes are produced by an autosomal allele (*s*) that is recessive to an allele for red eyes (s^+).

a. A female fly that has miniature wings and sepia eyes is crossed with a male that has normal wings and is homozygous for red

eyes. The F_1 flies are intercrossed to produce the F_2. Give the phenotypes, as well as their expected proportions, of the F_1 and F_2 flies.

b. A female fly that is homozygous for normal wings and has sepia eyes is crossed with a male that has miniature wings and is homozygous for red eyes. The F_1 flies are intercrossed to produce the F_2. Give the phenotypes, as well as their expected proportions, of the F_1 and F_2 flies.

41. Suppose that a recessive gene that produces a short tail in mice is located in the pseudoautosomal region. A short-tailed male mouse is mated with a female mouse that is homozygous for a normal tail. The F_1 mice from this cross are intercrossed to produce the F_2. Give the phenotypes, as well as their proportions, of the F_1 and F_2 mice.

42. *A color-blind woman and a man with normal color vision have three sons and six daughters. All the sons are color blind. Five of the daughters have normal color vision, but one of them is color blind. The color-blind daughter is 16 years old, is short for her age, and has not undergone puberty. Explain how this girl inherited her color blindness.

43. CONNECT Orange coat color in cats is an X-linked trait that is recessive to black. A black female cat had a father who was orange. How many copies of the orange allele will be present in the following cells in oogenesis of this black female? See Figure 2.20 for a review of oogenesis in animals.

a. primary oocyte

b. first polar body

c. ovum

Section 4.3

44. *How many Barr bodies would you expect to see in a human cell containing the following chromosomes?

    a. XX

    b. XY

    c. XO

    d. XXY

    e. XXYY

    f. XXXY

    g. XYY

    h. XXX

    i. XXXX

45. A woman with normal chromosomes mates with a man who also has normal chromosomes.

    a. Suppose that, in the course of oogenesis, the woman's sex chromosomes undergo nondisjunction in meiosis I; the man's chromosomes separate normally. Give all possible

combinations of sex chromosomes that this couple's children might inherit and the number of Barr bodies that you would expect to see in each of the cells of each child.

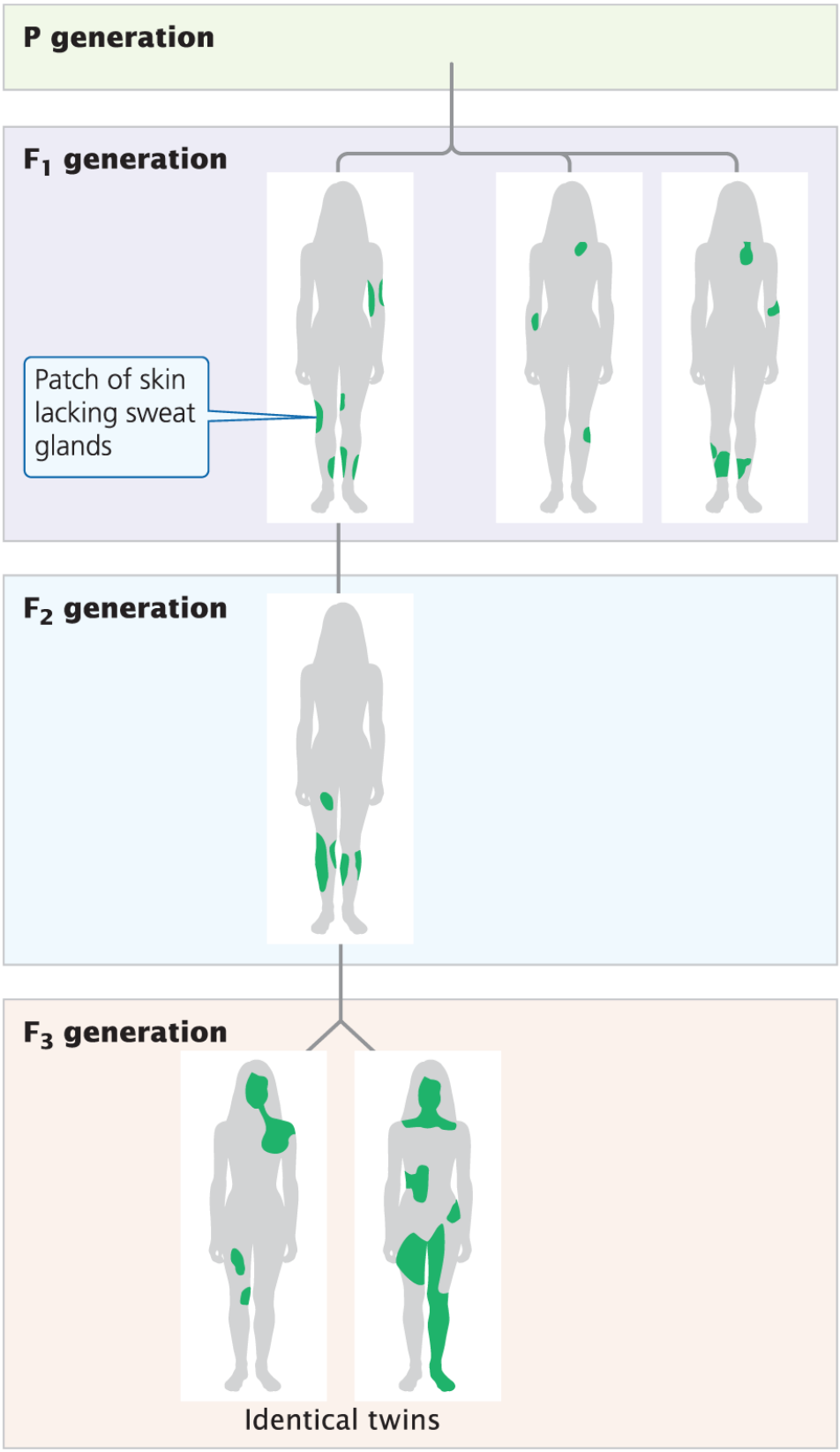
b. What chromosome combinations and numbers of Barr bodies would you expect to see if the chromosomes separate normally in oogenesis but nondisjunction of the sex chromosomes takes place in meiosis I of spermatogenesis?

46. What is the most likely sex and genotype of the cat shown in **Figure 4.19**?

47. *Hypohidrotic ectodermal dysplasia (also known as anhidrotic ectodermal dysplasia) is an X-linked recessive disorder in humans characterized by small teeth, no sweat glands, and sparse body hair. This trait is usually seen in men, but women who are heterozygous carriers of the trait often have irregular patches of skin with few or no sweat glands (see the illustration).

    a. Explain why women who are heterozygous carriers of a recessive gene for hypohidrotic ectodermal dysplasia have irregular patches of skin lacking sweat glands.

    b. Why does the distribution of the patches of skin lacking sweat glands differ among the females depicted in the illustration, even between the identical twins?



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Description
The illustration represents all the generations in different boxes. The boxes contain gray outlines of female figures with green patches on the figure representing areas of skin lacking sweat glands. The P generation on the top box is empty. The F subscript 1 generation below has 3 females. Female 1 has 5 small green patches on her legs and 2 on one arm. Female 2 has 1 on leg, one on arm and one on shoulder. Female 3 has 3 green patches on legs, 1 on arm and one on chest. Female 1 from the F subscript 1 generation has one female progeny in the F subscript 2 generation below. This female progeny has 5 green patches on her legs, which appear to be larger in size than the previous generation. The female from F subscript 2 generation has two female identical twins in F subscript 3 generation below. In twin 1, a relatively larger green patch is present on the face that extends to the shoulder and 2 small patches are on the leg. In twin 2, green patches appear on entire face, neck, collar bone area, almost an entire leg, top of the other leg, a spot on the hip and stomach.

CHALLENGE QUESTIONS

Section 4.2

48. CONNECT *A geneticist discovers a male mouse with greatly enlarged testes in his laboratory colony. He suspects that this trait results from a new mutation that is either Y linked or autosomal dominant. How could he determine whether the trait is autosomal dominant or Y linked? See Chapter 3 for a review of autosomal inheritance.

Section 4.3

49. Human females who are heterozygous for an X-linked recessive allele sometimes exhibit mild expression of the trait. However, such mild expression of X-linked traits in females who are heterozygous for X-linked alleles is not seen in *Drosophila.* What might cause this difference in the expression of X-linked genes between human females and female *Drosophila?* (Hint: In *Drosophila*, dosage compensation is accomplished by doubling the activity of genes on the X chromosome of males.)

50.

Identical twins (also called monozygotic twins) are derived from a single egg fertilized by a single sperm, creating a zygote that later divides into two (see Chapter 6). Because identical twins originate from a single zygote, they are genetically identical. Caroline Loat and her colleagues examined nine measures of social, behavioral, and cognitive ability in 1000 pairs of identical male twins and 1000 pairs of identical female twins (C. S. Loat et al. 2004. *Twin Research* 7:54–61). They found that for three of the measures (prosocial behavior, peer problems, and verbal ability), the two male twins of a pair tended to be more alike in their scores than were the two female twins of a pair. Propose a possible explanation for this observation. What might this observation indicate about the location of genes that influence prosocial behavior, peer problems, and verbal ability?

51. Occasionally, a mouse X chromosome is broken into two pieces and each piece becomes attached to a different autosome. In this event, the genes on only one of the two pieces undergo X

inactivation. What does this observation indicate about the mechanism of X-chromosome inactivation?

💬 ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 4.1

1. The duck-billed platypus has a unique mechanism of sex determination: females have five pairs of X chromosomes ($X_1X_1X_2X_2X_3X_3X_4X_4X_5X_5$) and males have five pairs of X and Y chromosomes ($X_1Y_1X_2Y_2X_3Y_3X_4Y_4X_5Y_5$). Do you think each of the X and Y chromosome pairs in males assorts independently of other X and Y pairs during meiosis? Why or why not?

2. Most organisms with XX-XY sex determination have pseudoautosomal regions, portions of the X and Y chromosomes that are homologous. Would you predict that organisms with ZZ-ZW sex determination have pseudoautosomal regions of homology between Z and W chromosomes? Explain your answer.

3. Both men and women produce testosterone, but concentrations of testosterone in the blood are generally higher in men than in women. However, the testosterone levels of some XX females fall within the range of testosterone levels of XY men. This overlap has created controversy within women's sports. Testosterone is known to increase muscle mass and enhance some types of athletic performance, so some people have suggested that women

with naturally high testosterone levels have an unfair competitive advantage. In 2011, the International Association of Athletics Federations (IAAF) adopted a policy that limited levels of testosterone in female athletes, saying that female athletes must not have a blood testosterone concentration greater than 10 nanomoles per liter (nmol/L), a level typically seen in men. Some elite female athletes have natural testosterone levels above this limit and have challenged the policy. Do you think that it is fair for XX females with naturally high testosterone (levels typically found in XY males) to compete in women's sports? Do they have an unfair advantage in competition with other women? What about male athletes with naturally high levels of testosterone? Do they have an unfair advantage over other males? In general, what role does genetics play in athletic competition—do some individuals have genes that give them an unfair advantage in competition?

Section 4.2

4. How is the inheritance of X-linked traits different from the inheritance of autosomal traits? How is the inheritance of X-linked and autosomal traits similar? List as many differences and similarities as you can.

5. On average, what proportion of X-linked genes in the first individual are the same (inherited from a common ancestor) as those in the second individual?

   a. A male and his mother

   b. A female and her mother

c. A male and his father

d. A female and her father

e. A male and his brother

f. A female and her sister

g. A male and his sister

h. A female and her brother

6. Red–green color blindness is an X-linked recessive trait. Susan has normal color vision, but her father is color blind. Susan mates with Bob, who has normal color vision.

   a. What is the probability that Susan and Bob will have a color-blind son?

   b. Susan and Bob have a daughter named Betty, who has normal color vision. If Betty mates with a man with normal color vision, and they have a son, what is the probability that the son will be color blind?

7. As pointed out in the section Evolution of the Y Chromosome, some researchers have predicted that the human Y chromosome will continue to lose genetic information in the future and will completely disappear from the species in about 10 million years. What would happen if the Y chromosome disappeared from the human species?

8. Hypohidrotic ectodermal dysplasia is an X-linked recessive disorder in humans characterized by small and reduced numbers

of teeth, no sweat glands, and sparse body hair (see Application Problems 29 and 47). The disorder is caused by mutations in the *EDA* gene, which produces a protein called ectodysplasin A1 (EDA1). In 2018, physicians reported the successful treatment of three boys with this disorder by administering synthetically produced EDA1 during in utero development at 26 to 31 weeks of gestation (H. Schneider, F. Faschingbauer, S. Schuepbach-Mallepell, et al. 2018. Prenatal correction of X-Linked hypohidrotic ectodermal dysplasia, *New England Journal of Medicine* 37:1604-1610). This treatment had a dramatic effect: after birth, the boys had normal sweating and increased numbers of teeth, although they still lacked hair. However, additional research found that administration of EDA1 after birth had no effect on boys with hypohidrotic ectodermal dysplasia. (a) Explain why treatment of EDA1 during intra uterine development produced a positive effect but treatment after birth had no effect. (b) Give a possible reason for development of hair was unaffected by the EDA1 treatment.

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Outline the mechanism of sex determination in central bearded dragons by creating a concept map using the following terms: central bearded dragon, ZZ males, ZW females, genetic sex determination, environmental sex determination, ZZ females.

Section 4.1

2. Create a concept map that displays the different ways that sex can be determined using the following terms: sex determination, environmental sex determination, XX-XO, ZZ-ZW, XX-XY, genic sex determination, chromosomal sex determination, male is heterogametic sex, female is heterogametic sex.

Section 4.2

3. Create a concept map that explains the mechanism of dosage compensation in mammals. Your concept map should include at least 10 concepts and connecting lines.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 5

Extensions and Modifications of Basic Principles

Courtesy of Dr. Reiko Kuroda.

The direction of shell coiling in *Lymnaea* snails is determined by genetic maternal effect. Shown here are two *Lymnaea stagnalis*: a snail with a left-handed (sinistral) shell on the left and a snail with a right-handed (dextral) shell on the right.

The Odd Genetics of Left-Handed Snails

At the start of the twentieth century, Mendel's work on inheritance in pea plants became widely known (see Chapter 3), and a number of biologists set out to verify his conclusions by conducting crosses with other organisms. Biologists quickly confirmed that Mendel's principles applied not just to peas but also to corn, beans, chickens, mice, guinea pigs, humans, and many other organisms. At the same time, biologists began to discover exceptions—traits whose inheritance was more complex than that of the simple dominant and recessive traits that Mendel had observed. One of these exceptions was the spiral of a snail's shell.

The direction of coiling in snail shells is called chirality. Most snail shells spiral downward in a clockwise or right-handed direction. These shells are said to be dextral. A few snails have shells that coil in the opposite direction, spiraling downward in a counterclockwise or left-handed direction. These shells are said to be sinistral. Most snail species have shells that are all dextral or all sinistral; only in a few rare instances do both dextral and sinistral shells coexist in the same species.

In the 1920s and 1930s, Arthur Boycott, of the University of London, investigated the genetics of shell coiling in *Lymnaea peregra*, a common pond snail in Britain. In this species, most snails are

dextral, but a few sinistral snails occur in some populations. Boycott learned from amateur naturalists of a pond near Leeds, England, where an abnormally high number of sinistral snails could be found. He obtained four sinistral snails from this location and began to investigate the genetics of shell chirality.

Boycott's research was complicated by the fact that these snails are hermaphroditic, meaning that a snail can self-fertilize, or *self* (mate with itself). If a suitable partner is available, the snails are also capable of *outcrossing*—mating with another individual. Boycott found that if he isolated a newly hatched snail and reared it alone, it would eventually produce offspring, so he knew that it had selfed. But when he placed two snails together and one produced offspring, he had no way of knowing whether it had mated with itself or with the other snail. Boycott's research required rearing large numbers of snails in isolation and in pairs, raising their offspring, and determining the direction of shell coiling for each offspring. To facilitate the work, he enlisted the aid of several amateur scientists. One of his helpers was Captain C. Diver, a friend who worked as an assistant for the British Parliament. Since Parliament met for only part of the year, Diver had time on his hands and eagerly enlisted to assist with the research. Together, Boycott, Diver, and other assistants carried out numerous breeding experiments, selfing and crossing snails and raising the progeny in jam jars. They eventually raised more than 6000 broods and determined the direction of coiling in a million snails.

Initially, their results were puzzling—shell coiling did not appear to conform to Mendel's principles of heredity. Eventually, they realized that dextral was dominant to sinistral but with a peculiar twist: the phenotype of a snail was determined *not* by its own genotype but by the genotype of its mother. This phenomenon—determination of the phenotype by the genotype of the mother—is called genetic maternal effect. Genetic maternal effect often arises because the maternal parent produces a substance, encoded by her own genotype, that is deposited in the cytoplasm of the egg and that influences early development of the offspring.

In 2016, several researchers found that direction of coiling in *Lymnaea* snails is determined by the *Lsdia1* gene, which plays a role in cytoskeleton formation. Sinistral snails possess a mutation in their *Lsdia1* alleles that prevents a functional protein from being produced. How the absence of this protein switches the direction of shell coiling is not yet clear.

THINK-PAIR-SHARE

- The introduction to this chapter discusses the genetic basis of chirality in snails and the research of Arthur Boycott, whose work established the mode of inheritance for this trait. In the course of his research, Boycott enlisted the aid of several amateur scientists—individuals who were not trained as scientists and had other jobs. The research would have been impossible without the aid of these people. In the past, amateur scientists such as these often made important contributions to science, but the practice is less frequent today. Discuss some possible reasons for the decline in contributions to research by amateur scientists. Are there any areas where amateur scientists still actively contribute?

- Genetic maternal effect is often seen in mammals. For example, research shows that the maternal genotype influences adult body size in mice. Why might these types of genetic effects be more common in mammals than in other organisms, such as fishes, amphibians, or reptiles?

Boycott's research on the direction of coiling in snails demonstrated that not all characteristics are inherited as simple dominant and recessive traits, like the shapes and colors of peas that Mendel described. This demonstration doesn't mean that Mendel was wrong; rather, it indicates that Mendel's principles are not, by themselves, sufficient to explain the inheritance of all genetic characteristics. Our modern understanding of genetics has been greatly enriched by the discovery of a number of modifications and extensions of Mendel's basic principles, which are the focus of this chapter.

5.1 Additional Factors at a Single Locus Can Affect the Results of Genetic Crosses

In Chapter 3, we learned that the principle of segregation and the principle of independent assortment enable us to predict the outcomes of genetic crosses. Here, we examine several additional factors acting at individual loci that can alter the phenotypic ratios predicted by Mendel's principles.

Types of Dominance

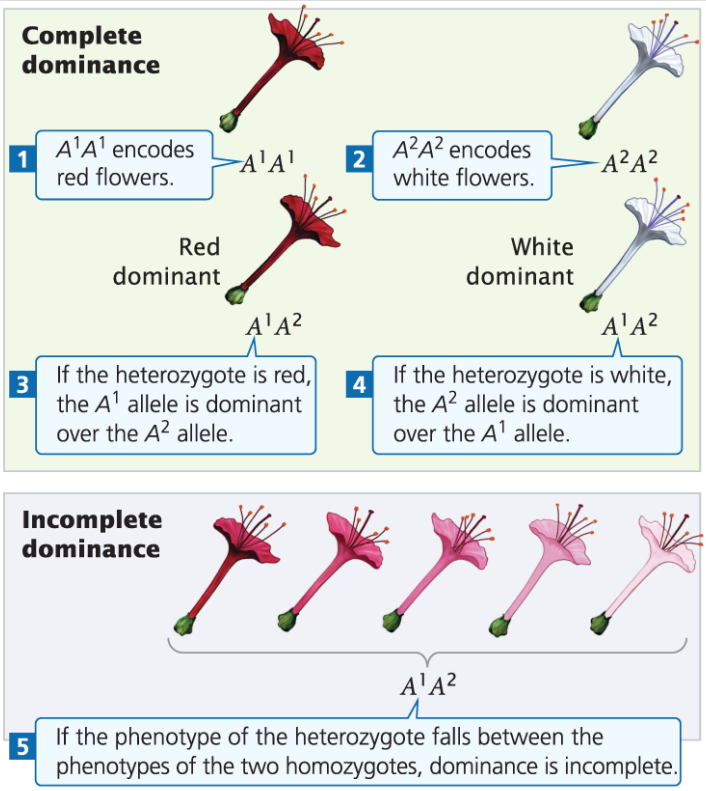
One of Mendel's important contributions to the study of heredity is the concept of dominance—the idea that although an individual organism possesses two different alleles for a characteristic, the trait encoded by only one of the alleles is observed in the phenotype. With dominance, the heterozygote possesses the same phenotype as one of the homozygotes.

Mendel observed dominance in all the traits he chose to study extensively, but he was aware that not all characteristics exhibit dominance. For example, he conducted some crosses to look at the length of time that pea plants take to flower. When he crossed two homozygous varieties that differed in their flowering time by an

average of 20 days, the length of time taken by the F_1 plants to flower was intermediate between those of the two parents. When the heterozygote has a phenotype intermediate between the phenotypes of the two homozygotes, the trait is said to display *incomplete dominance*.

COMPLETE AND INCOMPLETE DOMINANCE

Dominance can be understood in regard to how the phenotype of the heterozygote relates to the phenotypes of the two homozygotes. In the example presented in the upper panel of **Figure 5.1**, one homozygous genotype, A^1A^1, produces red pigment, resulting in red flowers; another, A^2A^2, produces no pigment, resulting in white flowers. The phenotype of the heterozygote determines the type of dominance. If the heterozygote (A^1A^2) produces the same amount of pigment as the A^1A^1 homozygote, resulting in red flowers, then the A^1 allele displays **complete dominance** over the A^2 allele; that is, red is dominant to white. If, on the other hand, the heterozygote produces no pigment, resulting in flowers with the same color as the A^2A^2 homozygote (white), then the A^2 allele is completely dominant, and white is dominant to red.



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5.1 The type of dominance exhibited by a trait depends on how the phenotype of the heterozygote relates to the phenotypes of the homozygotes.

Description

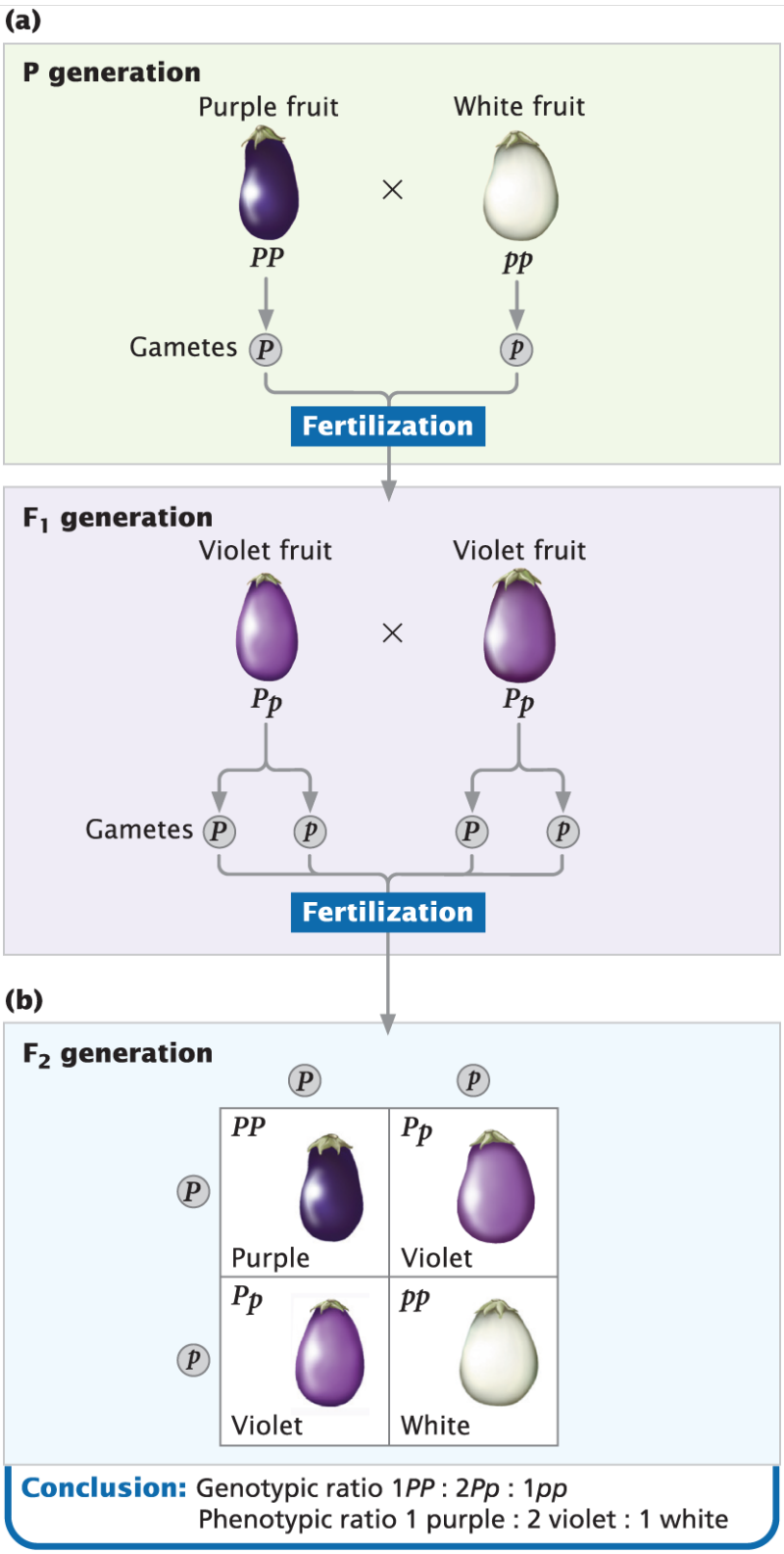
Complete dominance shows a red flower with genotype big A superscript 1 big A superscript 1 and a white flower with genotype big A superscript 2 big A superscript 2. The first caption corresponds to the red flower and reads, “big A superscript 1 big A superscript 1 encodes red flowers.” The second caption refers to the white flower and reads, “big A superscript 2 big A superscript 2 encodes white flowers.” Below the red flower, is a red flower labeled red dominant and below the white flower, is a white flower labeled white dominant. Both the flowers have the genotype big A superscript 1 big A superscript 2. The third caption below the red dominant flower and reads, “If the heterozygote is red, the big A superscript 1 allele is dominant over the big A superscript 2 allele.” The fourth caption below the white dominant flower and reads, “If the heterozygote is white, the big A superscript 2 allele is dominant over the big A superscript 1 allele.”

Five flowers are present in a series below that represents incomplete dominance. The color of flowers change from red to light pink gradually from left to right. All the flowers have the genotype big A superscript 1 big A superscript 2. The fifth caption reads, “If the phenotype of the heterozygote falls between the phenotypes of the two homozygotes, dominance is incomplete.”

When the phenotype of the heterozygote falls in between the phenotypes of the two homozygotes, dominance is incomplete. With **incomplete dominance**, the heterozygote need not be exactly intermediate between the two homozygotes (see the lower panel of Figure 5.1); it might be a slightly lighter shade of red or a slightly pink shade of white. As long as the heterozygote’s phenotype can be differentiated from those of the two homozygotes and falls between them, dominance is incomplete.

Incomplete dominance is also exhibited in the fruit color of eggplant. When a homozygous plant that produces purple fruit (*PP*) is crossed with a homozygous plant that produces white fruit (*pp*), all the heterozygous F_1 (*Pp*) plants produce violet fruit (**Figure 5.2a**). When the F_1 are crossed with each other, $^1/_4$ of the F_2 are purple (*PP*), $^1/_2$ are violet (*Pp*), and $^1/_4$ are white (*pp*), as shown in **Figure 5.2b**. Note that this 1:2:1 ratio is different from the 3:1 ratio that we would observe if eggplant fruit color exhibited complete dominance. Another example of incomplete dominance is feather color in chickens. A cross between a homozygous black chicken and a homozygous white chicken produces F_1 chickens that are gray. If

these gray F_1 chickens are intercrossed, they produce F_2 birds in a ratio of 1 black : 2 gray : 1 white.



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5.2 Fruit color in eggplant is inherited as an incompletely dominant trait.

Description
The part (a) shows P and F subscript 1 generation. In P generation, a purple eggplant with genotype big P big P is a crossed with a white eggplant with genotype small P small P. Gametes produced are big P and small P, respectively. Fertilization occurs and produces violet eggplants in the F subscript 1 generation shown below. Violet fruits with genotype big P small P are inter-crossed. Gametes produced are big P and small P for each plant. Fertilization takes place again and F subscript 2 generation is produced below in part (b).

The part (b) shows a Punnett square with 2 rows and 2 columns with gametes on its sides.

Row headers are as follows: big P and small P.

Column headers are as follows: big P and small P.

Row entries are depicted are as follows:

Row 1 column 1: big P big P (purple)

Row 1 column 2: big P small P (violet)

Row 2 column 1: big P small P (violet)

Row 2 column 2: small P small P (white)

A caption at the bottom reads, Conclusion: Genotypic ratio 1 big P big P to 2 big P small P to 1 small P small P. Phenotypic ratio 1 purple to 2 violet to 1 white.

We should now add the 1:2:1 ratio to the phenotypic ratios for simple crosses presented in Chapter 3 (see Table 3.5). A 1:2:1 phenotypic ratio arises in the progeny of a cross between two parents that are heterozygous for a characteristic that exhibits incomplete dominance ($Aa \times Aa$). The genotypic ratio among these progeny is also 1:2:1. When a trait displays incomplete dominance,

the genotypic ratios and phenotypic ratios of the offspring are the *same* because each genotype has its own phenotype. The important thing to remember about dominance is that it affects the way that genes are *expressed* (the phenotype) but not the way that genes are *inherited*.

CONCEPTS

Incomplete dominance is exhibited when the heterozygote has a phenotype intermediate between the phenotypes of the two homozygotes. When a trait exhibits incomplete dominance, a cross between two heterozygotes produces a 1:2:1 phenotypic ratio in the progeny.

✔ CONCEPT CHECK 1

If an F_1 eggplant in Figure 5.2 is used in a testcross, what proportion of the progeny from this cross will be white?

a. All the progeny
b. $^1/_2$
c. $^1/_4$
d. 0

CODOMINANCE

Another type of interaction between alleles is **codominance**. How is codominance different from incomplete dominance? In codominance, the phenotype of the heterozygote is not intermediate between the phenotypes of the homozygotes; rather, the

heterozygote simultaneously expresses the phenotypes of both homozygotes. An example of codominance is seen in the MN blood types of humans.

The MN blood-group locus encodes one of the types of antigens on the surface of red blood cells. Unlike foreign antigens of the ABO and Rh blood groups (the alleles of which encode red-blood-cell antigens), foreign MN antigens do not elicit a strong immunological reaction; therefore, the MN blood types are not routinely considered in blood transfusions. At the MN locus, there are two alleles: the L^{M} allele, which encodes the M antigen; and the L^{N} allele, which encodes the N antigen. Homozygotes with genotype $L^{M}L^{M}$ express the M antigen on the surface of their red blood cells and have the M blood type. Homozygotes with genotype $L^{N}L^{N}$ express the N antigen and have the N blood type. Heterozygotes with genotype $L^{M}L^{N}$ exhibit codominance and express both the M and the N antigens; they have blood type MN.

Some students might ask why the pink flowers illustrated in the lower panel of Figure 5.1 exhibit incomplete dominance—that is, why is this outcome not an example of codominance? The flowers would exhibit codominance only if the heterozygote produced both red and white pigments, which then combined to produce a pink phenotype. However, in our example, the heterozygote produces only red pigment. The pink phenotype comes about because the amount of pigment produced by the heterozygote is less than the amount produced by the $A^{1}A^{1}$ homozygote. So in that case, the

alleles clearly exhibit incomplete dominance, not codominance. The differences between complete dominance, incomplete dominance, and codominance are summarized in Table 5.1. ❯ TRY PROBLEM 13

TABLE 5.1 Differences between complete dominance, incomplete dominance, and codominance

Type of Dominance	Definition
Complete dominance	Phenotype of the heterozygote is the same as the phenotype of one of the homozygotes.
Incomplete dominance	Phenotype of the heterozygote is intermediate (falls within the range) between the phenotypes of the two homozygotes.
Codominance	Phenotype of the heterozygote includes the phenotypes of both homozygotes.

LEVEL OF PHENOTYPE OBSERVED MAY AFFECT DOMINANCE

Many phenotypes can be observed at several different levels, including the anatomical level, the physiological level, and the molecular level. The type of dominance exhibited by a characteristic

depends on the level at which the phenotype is examined. This dependency is seen in cystic fibrosis, a common genetic disorder in Caucasians that is usually considered to be a recessive disease. People who have cystic fibrosis produce large quantities of thick, sticky mucus, which plugs up the airways of the lungs and clogs the ducts leading from the pancreas to the intestine, causing frequent respiratory infections and digestive problems. Even with medical treatment, people with cystic fibrosis suffer chronic, life-threatening medical problems.

The gene responsible for cystic fibrosis resides on the long arm of chromosome 7. It encodes a protein termed *cystic fibrosis transmembrane conductance regulator* (CFTR), which acts as a gated channel in the cell membrane and regulates the movement of chloride ions into and out of the cell. People with cystic fibrosis have a mutated, dysfunctional form of CFTR that causes the channel to stay closed, so chloride ions build up in the cell. This buildup causes the formation of thick mucus and produces the symptoms of the disease.

Most people have two copies of the normal *CFTR* allele and produce only functional CFTR protein. Those with cystic fibrosis possess two mutated *CFTR* alleles and produce only the defective CFTR protein. Heterozygotes, who have one normal and one defective *CFTR* allele, produce both functional and defective CFTR protein. Thus, at the molecular level, the normal and defective *CFTR* alleles are codominant because both alleles are expressed in the heterozygote.

However, because one functional allele produces enough functional CFTR protein to allow normal chloride ion transport, heterozygotes exhibit no adverse effects, and the mutated *CFTR* allele appears to be recessive at the physiological level. The type of dominance expressed by an allele, as illustrated in this example, is thus a function of the phenotypic effect of the allele that we observe.

THINK-PAIR-SHARE Question 1

CHARACTERISTICS OF DOMINANCE

Several important characteristics of dominance should be emphasized. First, dominance is a result of interactions between genes at the same locus (allelic genes); in other words, dominance is *allelic* interaction. Second, dominance does not alter the way in which the genes are inherited; it influences only the way in which they are expressed as a phenotype. The allelic interaction that characterizes dominance is therefore interaction between the *products* of the genes. Finally, the type of dominance exhibited frequently depends on the level at which the phenotype is examined. As seen for cystic fibrosis, an allele may exhibit codominance at one level and be recessive at another level.

CONCEPTS

Dominance entails interactions between genes at the same locus (allelic genes) and is an aspect of the phenotype; dominance does not affect the way in which genes are inherited. The type of dominance exhibited by a characteristic frequently depends on the level at which the phenotype is examined.

✔ CONCEPT CHECK 2

How do complete dominance, incomplete dominance, and codominance differ?

Penetrance and Expressivity

In the genetic crosses presented thus far, we have considered only the interactions of alleles and have assumed that every individual organism having a particular genotype expresses the expected phenotype. We have assumed, for example, that in peas, the genotype *Rr* always produces round seeds and that the genotype *rr* always produces wrinkled seeds. For some characteristics, however, such an assumption is incorrect: the genotype does not always produce the expected phenotype, a phenomenon termed **incomplete penetrance**.

Incomplete penetrance is seen in human polydactyly, the condition of having extra fingers or toes (**Figure 5.3**). There are several different forms of human polydactyly, but the trait is usually caused by a dominant allele. Occasionally, people possess the allele for polydactyly (as evidenced by the fact that their children inherit the

polydactyly) but nevertheless have a normal number of fingers and toes. In these cases, the gene for polydactyly is not fully penetrant. **Penetrance** is defined as the percentage of individual organisms having a particular genotype that express the expected phenotype. For example, if we examined 42 people having an allele for polydactyly and found that only 38 of them were polydactylous, the penetrance would be ${}^{38}/_{42} = 0.90\ (90\%)$.

SPL/Science Source.

5.3 Human polydactyly (extra digits) exhibits incomplete penetrance and variable expressivity.

A related concept is that of **expressivity**, the degree to which a trait is expressed. In addition to incomplete penetrance, polydactyly exhibits variable expressivity. Some polydactylous people possess

extra fingers or toes that are fully functional, whereas others possess only a small tag of extra skin.

Incomplete penetrance and variable expressivity are due to the effects of other genes and environmental factors that can alter or completely suppress the effect of a particular gene. For example, a gene may encode an enzyme that produces a particular phenotype only within a limited temperature range. At higher or lower temperatures, the enzyme does not function, so the phenotype is not expressed; the allele encoding such an enzyme is therefore penetrant only within a particular temperature range (see also Environmental Effects on the Phenotype in Section 5.5). Many characteristics exhibit incomplete penetrance and variable expressivity; thus, the mere presence of a gene does not guarantee its expression. **› TRY PROBLEM 15**

CONCEPTS

Penetrance is the percentage of individuals having a particular genotype that express the associated phenotype. Expressivity is the degree to which a trait is expressed. Incomplete penetrance and variable expressivity result from the influence of other genes and environmental factors on the phenotype.

✔ CONCEPT CHECK 3

How does incomplete dominance differ from incomplete penetrance?

a. Incomplete dominance refers to alleles at the same locus; incomplete penetrance refers to alleles at different loci.

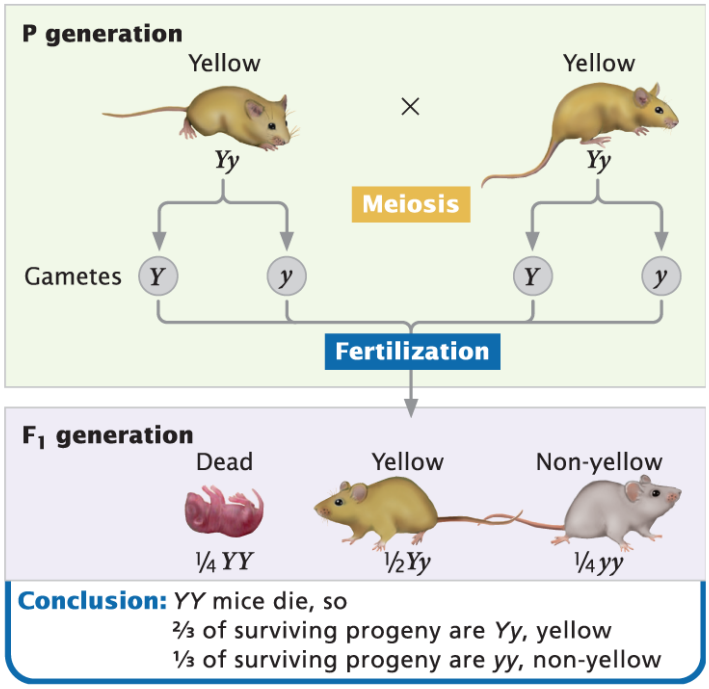
b. Incomplete dominance ranges from 0% to 50%; incomplete penetrance ranges from 51% to 99%.
c. In incomplete dominance, the heterozygote is intermediate between the homozygotes; in incomplete penetrance, heterozygotes express phenotypes of both homozygotes.
d. In incomplete dominance, the heterozygote is intermediate between the homozygotes; in incomplete penetrance, some individuals do not express the expected phenotype.

Lethal Alleles

A **lethal allele** causes death at an early stage of development—often before birth—so that some genotypes do not appear among the progeny. An example of a lethal allele, originally described by Erwin Baur in 1907, is found in snapdragons. The *aurea* strain in these plants has yellow leaves. When two plants with yellow leaves are crossed, $^2/_3$ of the progeny have yellow leaves and $^1/_3$ have green leaves. When green is crossed with green, all the progeny have green leaves; when yellow is crossed with green, however, $^1/_2$ of the progeny have green leaves and $^1/_2$ have yellow leaves, confirming that all yellow-leaved snapdragons are heterozygous.

Another example of a lethal allele is one that determines yellow coat color in mice. A cross between two yellow heterozygous mice produces an initial genotypic ratio of $^1/_4$ *YY*, $^1/_2$ *Yy*, and $^1/_4$ *yy*, but the homozygous *YY* mice die early in development and do not appear among the progeny, resulting in a 2:1 ratio of *Yy* (yellow) to *yy* (non-yellow) in the offspring (**Figure 5.4**). A 2:1 ratio is almost always

produced by a recessive lethal allele, so observing this ratio among the progeny of a cross between individuals with the same phenotype is a strong clue that one of the alleles is lethal. In this example, as in that of yellow leaves in snapdragons, the lethal allele (*Y*) is recessive because it causes death only in homozygotes. Unlike its effect on *survival*, the effect of the yellow allele on *color* is dominant; in both mice and snapdragons, a single copy of the allele in heterozygotes produces a yellow color. These examples illustrate the point made earlier (pp. 114–115) that the type of dominance depends on the aspect of the phenotype examined.



5.4 The 2:1 ratio produced by a cross between two yellow mice results from a lethal allele.

Description

Two yellow mice each with genotype big Y small Y are crossed in generation P and each produce 2 gametes; an big Y and an small Y. Fertilization occurs and results in one-fourth dead mice of genotype big Y big Y, one-half yellow mice of genotype big Y small Y, and one-fourth non-yellow mice of genotype small Y small Y in the F subscript 1 generation shown below.

A caption at the bottom reads, “Conclusion: big Y big Y mice die, so two-third of surviving progeny are big Y small Y, yellow; one-third of surviving progeny are small Y small Y, non-yellow.”

Many lethal alleles in nature are recessive, but lethal alleles can also be dominant; in this case, homozygotes and heterozygotes for the allele die. Truly dominant lethal alleles cannot be transmitted unless they are expressed after the onset of reproduction. › **TRY** PROBLEM 17

CONCEPTS

A lethal allele causes death at an early developmental stage, so that one or more genotypes are missing from the progeny of a cross. Lethal alleles therefore modify the ratio of progeny resulting from a cross.

✔ CONCEPT CHECK 4

A cross between two green corn plants yields $^{2}/_{3}$ progeny that are green and $^{1}/_{3}$ progeny that are yellow. What is the genotype of the green progeny?

a. *WW*
b. *Ww*
c. *ww*
d. *W*_(*WW* and *Ww*)

Multiple Alleles

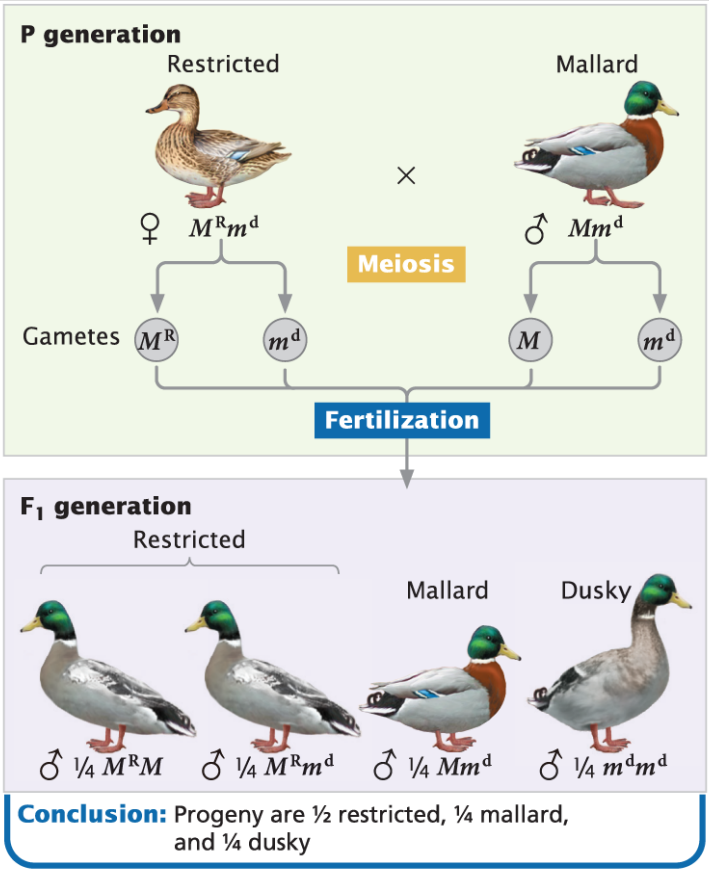
Most of the genetic systems that we have examined so far consist of two alleles. In Mendel's peas, for instance, one allele encoded round seeds and another encoded wrinkled seeds; in cats, one allele produced a black coat and another produced a gray coat. For some loci, however, more than two alleles are present within a group of organisms—the locus has **multiple alleles** (which may also be referred to as an *allelic series*). Although there may be more than two alleles present within a *group* of organisms, the genotype of each individual diploid organism still consists of only two alleles. The inheritance of characteristics encoded by multiple alleles is no different from the inheritance of characteristics encoded by two alleles except that a greater variety of genotypes and phenotypes are possible.

PLUMAGE PATTERNS IN DUCKS

An example of multiple alleles is found at a locus that determines the plumage pattern of mallard ducks. One allele, M, produces the wild-type *mallard* pattern. A second allele, M^{R} produces a different pattern called *restricted*, and a third allele, m^{d}, produces a pattern termed *dusky*. In this allelic series, restricted is dominant to mallard and dusky, and mallard is dominant to dusky: $M^{R} > M > m^{d}$. The six genotypes possible with these three alleles, and their resulting phenotypes, are

Genotype	Phenotype
$M^R M^R$	restricted
$M^R M$	restricted
$M^R m^d$	restricted
MM	mallard
Mm^d	mallard
$m^d m^d$	dusky

In general, the number of genotypes possible will be $[n(n + 1)/2]$, where n equals the number of different alleles at a locus. Working crosses with multiple alleles is no different from working crosses with two alleles; Mendel's principle of segregation still holds, as shown in the cross between a restricted duck and a mallard duck (Figure 5.5). › **TRY** PROBLEM 19



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5.5 Mendel's principle of segregation applies to crosses with multiple alleles. In this example, three alleles determine the type of plumage in mallard ducks: M^R (restricted) > M (mallard) > m^d (dusky). Note that the phenotypes of male and female mallard ducks differ. The restricted pattern of a female duck is shown for the female parent. All the progeny in the F_1 have male plumage patterns.

Description

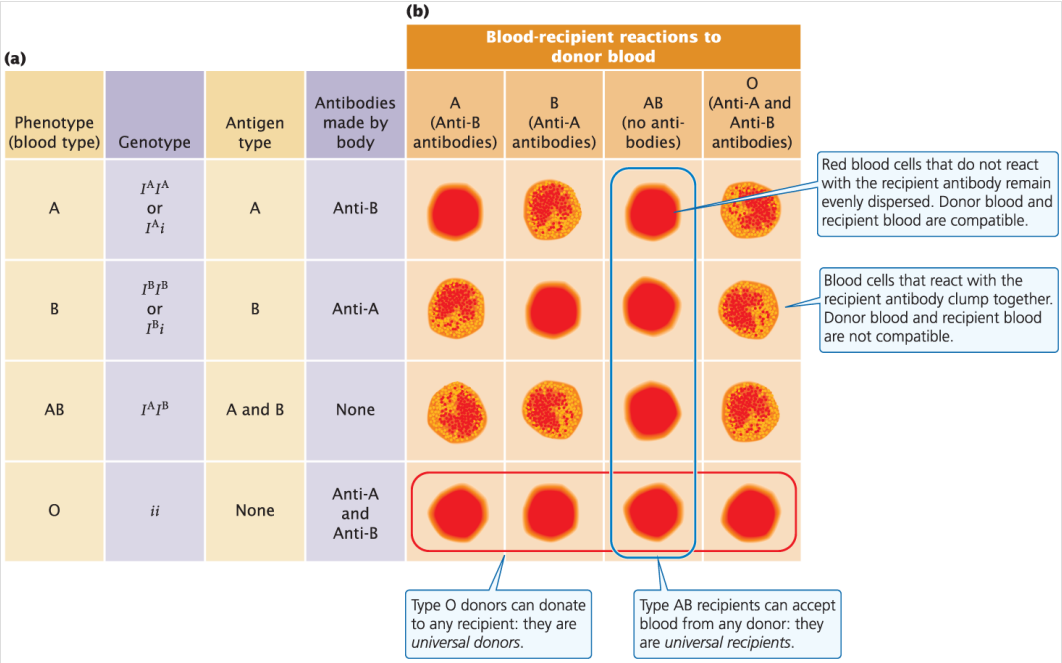
In generation P, a restricted female duck with genotype big M superscript big R small M superscript small D is crossed with a male mallard with genotype big M small M superscript small D. Gametes produced are as follows: big M superscript big R and small M superscript small D from female duck and big M and small M superscript small D from male duck. Fertilization occurs and the F subscript 1 generation is obtained below. The F subscript 1 generation shows one-fourth male with genotype big M superscript big R big M (restricted), one-fourth male with genotype big M superscript big R small M superscript

small D (restricted), one-fourth male with genotype big M small M superscript small D (mallard), and one-fourth male with genotype small M superscript small D small M superscript small D (dusky). A caption at the bottom reads: Progeny are one half restricted, one-fourth mallard, and one-fourth dusky.

THE ABO BLOOD GROUP IN HUMANS

Another multiple-allele system is found at the locus for the ABO blood group, which determines your ABO blood type. What do you know about ABO blood types?

The ABO locus, like the MN locus, encodes antigens on the surface of red blood cells. The three common alleles for the ABO blood group locus are I^A, which encodes the A antigen; I^B, which encodes the B antigen; and i, which encodes no antigen (O). We can represent the dominance relations among the ABO alleles as follows: $I^A > i$, $I^B > i$, $I^A = I^B$. The I^A and the I^B alleles are dominant to i and are codominant with each other; the AB phenotype is due to the presence of an I^A allele and an I^B allele, which results in the production of A and B antigens on red blood cells. A person with genotype ii produces neither antigen and has blood type O. The six common genotypes at this locus and their phenotypes are shown in Figure 5.6a.



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5.6 ABO blood types and the outcomes of possible blood transfusions.

Description

Table (a) has four columns and four rows. The column headers are as follows: Phenotype (blood type), Genotype, Antigen type, and Antibodies made by body.

The row entries for table (a) are as follows:

Row 1: Blood type, A; genotype, big I superscript A big I superscript A or big I superscript big A small I; antigen type, A; antibodies made by body, Anti-B.

Row 2: Blood type, B; genotype, big I superscript B big I superscript B or big I superscript big B small I; antigen type, B; antibodies made by body, Anti-A.

Row 3: Blood type, A B; genotype, big I superscript A big I superscript B; antigen type, A and B; antibodies made by body, none.

Row 4: Blood type, O; genotype, small I small I; antigen type, none; antibodies made by body, Anti-A and Anti-B.

Table (b) titled “blood recipient reactions to donor blood” has four columns and four rows.

The column headers for table (b) are as follows: A (Anti-B antibodies), B (Anti-A antibodies), A B (no antibodies), and O (Anti-A and Anti-B antibodies).

The row entries for table (b) are as follows:

Row 1: For blood type A; 1, evenly dispersed red blood cells; 2, clumps of red blood cells; 3, evenly dispersed red blood cells; 4, clumps of red blood cells.

Row 2: For blood type B; 1, clumps of red blood cells; 2, evenly dispersed red blood cells; 3, evenly dispersed red blood cells; 4, clumps of red blood cells.

Row 3: For blood type A B; 1, clumps of red blood cells; 2, clumps of red blood cells; 3, evenly dispersed red blood cells; 4, clumps of red blood cells.

Row 4: For blood type O; 1, evenly dispersed red blood cells; 2, evenly dispersed red blood cells; 3, evenly dispersed red blood cells; 4, evenly dispersed red blood cells.

A rectangular boundary is formed around the third column and the caption besides reads: Red blood cells that do not react with the recipient antibody remain evenly dispersed. Donor blood and recipient blood are compatible. A rectangular boundary is formed around the fourth row and the caption besides reads: Type O donors can donate to any recipient: they are universal donors. The region where both the boundaries intersect that is the fourth entry in third column and third entry of fourth column has a caption that reads: Type A B recipients can accept blood from any donor: they are universal recipients.

A caption referring to the fourth entry of the second row reads: Blood cells that react with the recipient antibody clump together. Donor blood and recipient blood are not compatible.

The body produces antibodies against any foreign antigens (see Figure 5.6a). For instance, a person with blood type A produces anti-B antibodies because the B antigen is foreign to that person. A person with blood type B produces anti-A antibodies, and a person with blood type AB produces neither anti-A nor anti-B antibodies because neither the A nor the B antigen is foreign to that person. A person with blood type O possesses no A or B antigens;

consequently, that person produces both anti-A antibodies and anti-B antibodies. The presence of antibodies against foreign ABO antigens means that successful blood transfusions are possible only between people with certain compatible blood types (**Figure 5.6b**).

The inheritance of alleles at the ABO locus is illustrated by a paternity suit against the movie actor Charlie Chaplin. In 1941, Chaplin met a young actress named Joan Barry, with whom he had an affair. The affair ended in February 1942, but 20 months later, Barry gave birth to a baby girl and claimed that Chaplin was the father. Barry then sued for child support. At this time, blood typing had just come into widespread use, and Chaplin's attorneys had Chaplin, Barry, and the child blood typed. Barry had blood type A, her child had blood type B, and Chaplin had blood type O. Could Chaplin have been the father of Barry's child?

Your answer should be no. Joan Barry had blood type A, which can be produced by either genotype I^AI^A or genotype I^Ai. Her baby possessed blood type B, which can be produced by either genotype I^BI^B or genotype I^Bi. The baby could not have inherited the I^B allele from Barry (Barry could not carry an I^B allele if she were blood type A); therefore, the baby must have inherited the i allele from her. Barry must have had genotype I^Ai, and the baby must have had genotype I^Bi. Because the baby girl inherited her i allele from Barry, she must have inherited the I^B allele from her father. Having blood type O, produced only by genotype ii, Chaplin could not have been

the father of Barry's child. While blood types can be used to exclude the possibility of paternity (as in this case), they cannot prove that a particular person is the parent of a child because many different people have the same blood type.

In the course of the trial to settle the paternity suit against Chaplin, three pathologists testified that it was genetically impossible for Chaplin to have fathered the child. Nevertheless, the jury ruled that Chaplin was the father and ordered him to pay child support and Barry's legal expenses. **› TRY PROBLEM 24**

COMPOUND HETEROZYGOTES

Different alleles often give rise to the same phenotype. For example, cystic fibrosis, as we saw earlier in this section, arises from defects in alleles at the *CFTR* locus, which encodes a protein that controls the movement of chloride ions into and out of the cell. Over a thousand different alleles at the *CFTR* locus that can cause cystic fibrosis have been discovered worldwide. Because cystic fibrosis is an autosomal recessive condition, one must normally inherit two defective *CFTR* alleles to have cystic fibrosis. In some people with cystic fibrosis, these two defective alleles are identical, meaning that the person is homozygous. Other people with cystic fibrosis are heterozygous, possessing two different defective alleles. An individual who carries two different alleles at a locus that result in a recessive phenotype is referred to as a **compound heterozygote**.

CONCEPTS

More than two alleles (multiple alleles) may be present at a locus within a group of organisms, although each individual diploid organism still has only two alleles at that locus. A compound heterozygote possesses two different alleles that result in a recessive phenotype.

✔ CONCEPT CHECK 5

How many genotypes are possible at a locus with five alleles?

a. 30
b. 27
c. 15
d. 5

5.2 Gene Interaction Takes Place When Genes at Multiple Loci Determine a Single Phenotype

In the dihybrid crosses that we examined in Chapter 3, each locus had an independent effect on the phenotype. When Mendel crossed a homozygous pea plant that produced round and yellow seeds (*RR YY*) with a homozygous plant that produced wrinkled and green seeds (*rr yy*), and then allowed the F_1 to self-fertilize, he obtained F_2 progeny in the following proportions:

$^9/_{16}\ R_Y_$	round, yellow
$^3/_{16}\ R_yy$	round, green
$^3/_{16}\ rr\ Y_$	wrinkled, yellow
$^1/_{16}\ rr\ yy$	wrinkled, green

In this example, the two loci showed two kinds of independence. First, the genes at each locus were independent in their *assortment* in meiosis, which produced the 9:3:3:1 ratio of phenotypes in the progeny, in accord with Mendel's principle of independent assortment. Second, the genes were independent in their *phenotypic expression*: the *R* and *r* alleles affected only the shape of the seed and had no influence on the color of the seed; the *Y* and *y* alleles affected only color and had no influence on the shape of the seed.

Frequently, genes exhibit independent assortment but do not act independently in their phenotypic expression; instead, the effects of genes at

one locus depend on the presence of genes at other loci. This type of interaction between the effects of genes at different loci (genes that are not allelic) is termed **gene interaction**. With gene interaction, it is the products of genes at different loci that interact to produce new phenotypes that are not predictable from the single-locus effects alone. In our consideration of gene interaction, we will focus primarily on interactions between the effects of genes at two loci, although interactions among genes at three, four, or more loci are common.

CONCEPTS

In gene interaction, genes at different loci contribute to the determination of a single phenotypic characteristic.

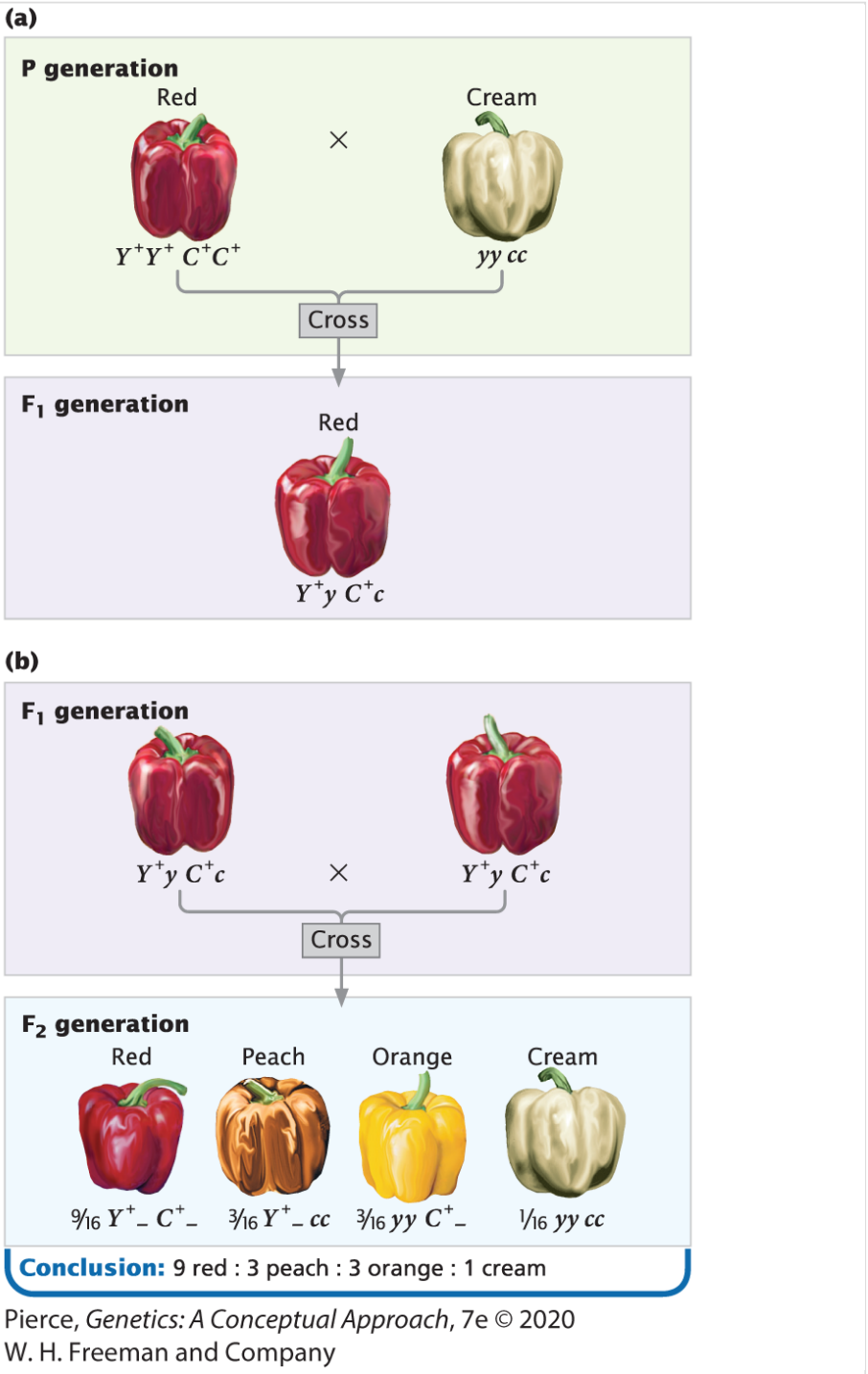
✔ CONCEPT CHECK 6

How does gene interaction differ from dominance?

Gene Interaction That Produces Novel Phenotypes

Let's begin by examining gene interaction in which genes at two loci interact to produce a single characteristic. Fruit color in the pepper *Capsicum annuum* is determined in this way. Certain types of peppers produce fruits in one of four colors: red, peach, orange (sometimes called yellow), or cream (white). If a homozygous plant with red peppers is crossed with a homozygous plant with cream peppers, all the F_1 plants have red peppers (**Figure 5.7a**). When the F_1 are crossed with each other, the F_2 show a ratio of 9 red : 3 peach : 3 orange : 1 cream (**Figure 5.7b**). This dihybrid ratio (see Chapter 3) is

produced by a cross between two plants that are both heterozygous for two loci ($Y^+y\ C^+c \times Y^+y\ C^+c$). In this example, the *Y* locus and the *C* locus interact to produce a single phenotype—the color of the pepper:



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5.7 Interaction between genes at two loci determines a single characteristic, fruit color, in the pepper *Capsicum annuum*.

Description
The part a shows P generation with the following genotype: big Y superscript positive big Y superscript positive (red) and small Y small Y small C small C (cream). F subscript 1 generation below produced after cross shows the following genotype: big Y superscript positive small Y big C superscript positive small C (red). The part b shows F subscript 1 generation where two red peppers with same genotype big Y superscript positive small Y big C superscript positive small C are crossed to produce F subscript 2 generation. The F subscript 2 generation shown below has the following genotype: nine over sixteen Y superscript positive underscore C superscript positive underscore (red), three over sixteen big Y superscript positive underscore small C small C (peach), three over sixteen small Y small Y big C superscript positive underscore (orange), and one over sixteen small Y small Y small C small C (cream). A caption below reads, Conclusion: 9 red to 3 peach to 3 orange to 1 cream.

Genotype	Phenotype
$Y^+_\ C^+_$	Red
$Y^+_\ cc$	Peach
$yy\ C^+_$	Orange
$yy\ cc$	Cream

To illustrate how Mendel's rules of heredity can be used to understand the inheritance of characteristics determined by gene interaction, let's consider a testcross between an F_1 plant from the cross in Figure 5.7 ($Y^+y\ C^+c$) and a plant with cream peppers ($yy\ cc$). As outlined in Chapter 3 for independent loci, we can work this cross by breaking it down into two simple crosses. At the first locus, the heterozygote Y^+y is crossed with the homozygote yy; this cross produces $^1/_2\ Y^+y$ and $^1/_2\ yy$ progeny. Similarly, at the second locus, the

heterozygous genotype C^+c is crossed with the homozygous genotype *cc*, producing $^1/_2$ C^+c and $^1/_2$ *cc* progeny. In accord with Mendel's principle of independent assortment, these single-locus ratios can be combined by using the multiplication rule: the probability of obtaining the genotype Y^+y C^+c is the probability of Y^+y $(^1/_2)$ multiplied by the probability of C^+c $(^1/_2)$, or $^1/_2 \times {}^1/_2 = {}^1/_4$. The probabilities of the progeny genotypes resulting from the testcross are

Progeny genotype	Probability at each locus		Overall probability	Phenotype
Y^+y C^+c	$^1/_2 \times {}^1/_2$	=	$^1/_4$	red peppers
Y^+y *cc*	$^1/_2 \times {}^1/_2$	=	$^1/_4$	peach peppers
yy C^+c	$^1/_2 \times {}^1/_2$	=	$^1/_4$	orange peppers
yy cc	$^1/_2 \times {}^1/_2$	=	$^1/_4$	cream peppers

When you work problems involving gene interaction, it is especially important to determine the probabilities of single-locus genotypes and to multiply the probabilities of *genotypes,* not phenotypes, because the phenotypes cannot be determined without considering the effects of the genotypes at all the contributing loci. **› TRY PROBLEM 25**

Gene Interaction with Epistasis

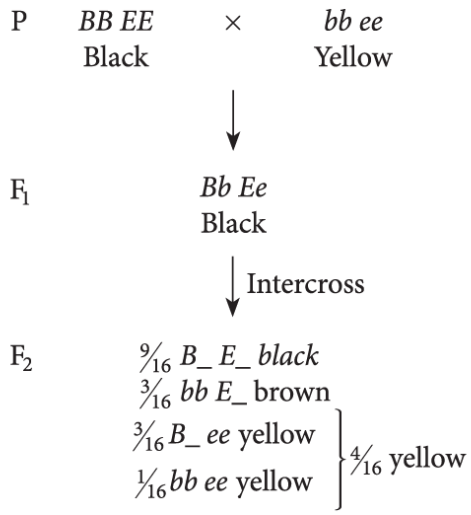
Sometimes the effect of gene interaction is that one gene masks (hides) the effect of another gene at a different locus, a phenomenon known as **epistasis**. In the examples of gene interaction that we have already examined, genes at different loci interacted to determine a single phenotype, but one gene did not *mask* the effect of a gene at another locus, meaning that there was no epistasis. Epistasis is similar to dominance, except that dominance entails the masking of genes at the *same* locus (allelic genes). In epistasis, the gene that does the masking is called an **epistatic gene**; the gene whose effect is masked is a **hypostatic gene**. Epistatic genes may be recessive or dominant in their effects.

RECESSIVE EPISTASIS

Recessive epistasis is seen in the genes that determine coat color in Labrador retrievers. These dogs may be black, brown (frequently called chocolate), or yellow; their different coat colors are determined by interactions between genes at two loci (although a number of other loci also help to determine coat color; see pp. 126–128). One locus determines the type of pigment produced by the skin cells: a dominant allele *B* encodes black pigment, whereas a recessive allele *b* encodes brown pigment. Alleles at a second locus affect the *deposition* of the pigment in the shaft of the hair; dominant allele *E* allows dark pigment (black or brown) to be deposited, whereas recessive allele *e* prevents the deposition of dark pigment, causing the hair to be yellow. The presence of genotype *ee* at the second locus therefore masks the expression of the black and brown alleles at the first locus. The genotypes that determine coat color and their phenotypes are

Genotype	Phenotype
*B_ E_*	Black
*bb E_*	Brown
B_ ee	Yellow
bb ee	Yellow

If we cross a black Labrador that is homozygous for the dominant alleles (*BB EE*) with a yellow Labrador that is homozygous for the recessive alleles (*bb ee*) and then intercross the F_1, we obtain progeny in the F_2 in a 9:3:4 ratio:

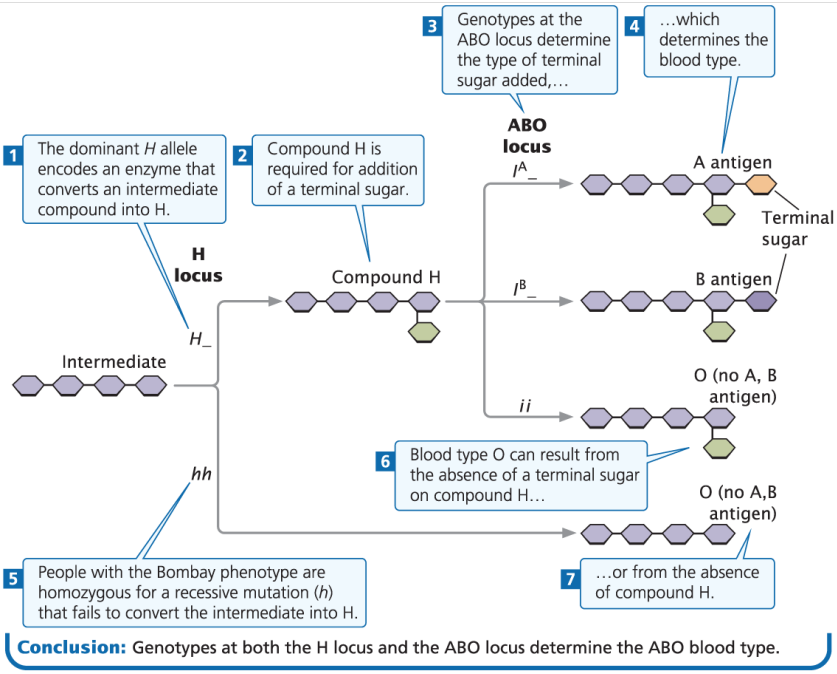


Description
The P generation shows black Labrador with genotype big B big B big E big E crossed with yellow Labrador with genotype small B small B small E small E. The F subscript 1 generation below shows black progeny with genotype big B small B big E small E. Intercross results in F subscript 2 generation below which consists of nine over sixteen big B blank big E blank, black; three over sixteen small B small B big E blank, brown; three over sixteen big B blank small E small E, yellow; one over sixteen small B small B, small E small E, yellow. Four over sixteen progeny in F subscript 2 generation are yellow.

Notice that yellow dogs can carry alleles for either black or brown pigment, but these alleles are not expressed in their coat color.

In this example of gene interaction, allele *e* is epistatic to *B* and *b* because *e* masks the expression of the alleles for black and brown pigments, and alleles *B* and *b* are hypostatic to *e*. In this case, *e* is a recessive epistatic allele because two copies of *e* must be present to mask the expression of the black and brown pigments. **› TRY PROBLEM 29**

Another recessive epistatic gene determines a rare blood type called the Bombay phenotype; this gene masks the expression of alleles at the ABO locus. As mentioned earlier in the chapter, the alleles at the ABO locus encode antigens on the red blood cells; the antigens consist of short chains of carbohydrates embedded in the outer membranes of red blood cells. The difference between the A and the B antigens is a function of chemical differences in the terminal sugar of the chain. The I^A and I^B alleles actually encode different enzymes, which add sugars designated A or B to the ends of the carbohydrate chains (**Figure 5.8**). The common substrate on which these enzymes act is a molecule called compound H. In individuals with blood type O, either the enzyme encoded by the *i* allele adds no sugar to compound H or no functional enzyme is specified.



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5.8 Expression of the ABO antigens depends on alleles at the H locus. The H locus encodes a precursor to the antigens, called compound H. Alleles at the ABO locus determine which types of terminal sugars are added to compound H.

Description

The branch diagram involves 3 steps and the process starts with an intermediate and divides into two branches based on big H locus through big H blank and small H small H. A caption reads, “The dominant big H allele encodes an enzyme that converts an intermediate compound into H.” Through big H blank, compound big H is formed on the right side and shows one added molecule at terminal position in the intermediate. A caption reads, “Compound H is required for addition of a terminal sugar.” Compound H branches into three divisions based on big A big B big O locus. A caption reads, “Genotypes at the big A big B big O locus determine the type of terminal sugar added which determines the blood type.” The first branch produces big A-antigen with a terminal sugar in compound big H through big I superscript A blank. The second branch produces big B-antigen with a terminal sugar in compound big H through big I superscript B blank. The third branch produces big O (no big A, big B antigen) with no added terminal sugar in compound big H through small I small I. The second branch from the intermediate directly leads to big O blood type through small H small H without the inclusion of compound big H. A caption from small H small H reads, “People with the Bombay phenotype are homozygous for a recessive mutation (small h) that fails to convert the intermediate into big H.” A caption from blood type big O reads, “Blood-type big O can result from the absence of a terminal sugar on compound big H or from the absence of compound big H.” A caption at

the bottom reads, “Conclusion: Genotypes at both the big H locus and the big A big B big O locus determine the big A big B big O blood type.”

In most people, a dominant allele (*H*) at the H locus encodes an enzyme that makes compound H, but people with the Bombay phenotype are homozygous for a recessive mutation (*h*) that encodes a defective enzyme. The defective enzyme is incapable of making compound H, and because compound H is not produced, no ABO antigens are synthesized. Thus, the expression of the alleles at the ABO locus depends on the genotype at the H locus.

Genotype	Compound H present	ABO phenotype
*H_* I^AI^A, *H_* I^Ai	Yes	A
*H_* I^BI^B, *H_* I^Bi	Yes	B
*H_* I^AI^B	Yes	AB
H_ii	Yes	O
hh I^AI^A, *hh* I^Ai, *hh* I^BI^B, *hh* I^Bi, *hh* I^AI^B, and *hh ii*	No	O

In this example, the alleles at the ABO locus are hypostatic to the recessive *h* allele.

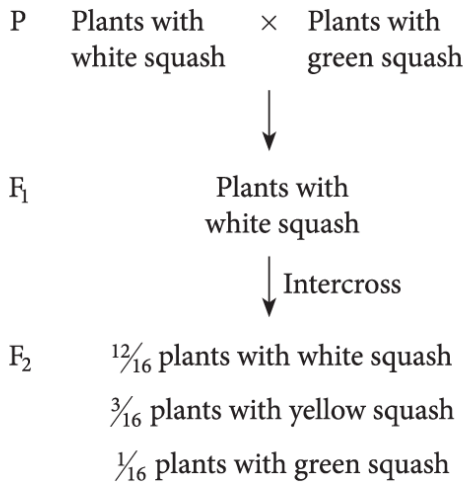
The Bombay phenotype provides us with a good opportunity for considering how epistasis can arise when genes affect a series of steps in a biochemical pathway. The ABO antigens are produced in a multistep biochemical pathway

(see Figure 5.8) that depends on enzymes that make compound H and on other enzymes that convert compound H into the A or B antigen. Note that blood type O may arise in one of two ways: (1) from failure to add a terminal sugar to compound H (genotype *H_ii*) or (2) from failure to produce compound H (genotype *hh_*). Many cases of epistasis arise in similar ways. A gene (such as *h*) that has an effect on an early step in a biochemical pathway will be epistatic to genes (such as I^A and I^B) that affect subsequent steps because the effects of the genes in a later step depend on the product of the earlier reaction.

DOMINANT EPISTASIS

In *recessive* epistasis, the presence of two recessive alleles (the homozygous genotype) inhibits the expression of an allele at a different locus. In *dominant* epistasis, however, only a single copy of an allele is required to inhibit the expression of an allele at a different locus.

Dominant epistasis is seen in the interaction of two loci that determine fruit color in summer squash, which is commonly found in one of three colors: yellow, white, or green. When a homozygous plant that produces white squash is crossed with a homozygous plant that produces green squash and the F_1 plants are crossed with each other, the following results are obtained:



Description
The P generation shows plants with white squash crossed with plants with green squash. Fertilization occurs and F subscript 1 generation is obtained below. F subscript 1 generation has plants with white squash. Intercross produces F subscript 2 generation below which has twelve-sixteenth plants with white squash, three-sixteenth plants with yellow squash, and one-sixteenth plants with green squash.

How can gene interaction explain these results?

In the F_2, $^{12}/_{16}$, or $^{3}/_{4}$, of the plants produce white squash, and $^{3}/_{16} + {}^{1}/_{16} = {}^{4}/_{16} = {}^{1}/_{4}$ of the plants produce colored squash. This outcome is the familiar 3:1 ratio produced by a cross between two heterozygotes, which suggests that a dominant allele at one locus inhibits the production of pigment, resulting in white progeny. If we use the symbol *W* to represent the dominant allele that inhibits pigment production, then genotype *W*_ inhibits pigment production and produces white squash, whereas *ww* allows pigment production and results in colored squash.

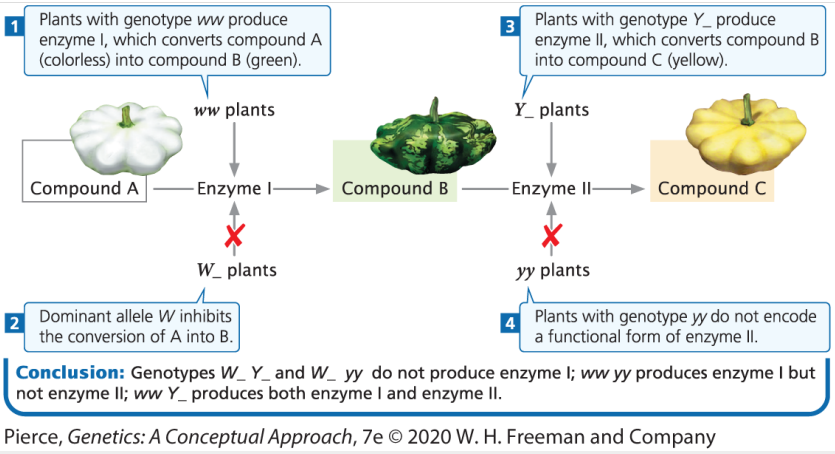
Among those *ww* F_2 plants with pigmented fruit, we observe $^{3}/_{16}$ yellow and $^{1}/_{16}$ green (a 3:1 ratio). This observation suggests that a second locus determines the type of pigment produced in the squash, with yellow (*Y*_) dominant to green (*yy*). This locus is expressed only in *ww* plants, which lack

the dominant inhibitory allele *W*. We can assign the genotype *ww Y_* to plants that produce yellow squash and the genotype *ww yy* to plants that produce green squash. The genotypes and their associated phenotypes are

*W_ Y_*	white squash
W_ yy	white squash
*ww Y_*	yellow squash
ww yy	green squash

Allele *W* is epistatic to *Y* and *y*: it masks the expression of these pigment-producing genes. Allele *W* is a dominant epistatic allele because, in contrast with *e* in Labrador retriever coat color and with *h* in the Bombay phenotype, a single copy of the allele is sufficient to inhibit pigment production.

Yellow pigment in the squash is most likely produced in a two-step biochemical pathway (**Figure 5.9**). A colorless (white) compound (designated A in Figure 5.9) is converted by enzyme I into green compound B, which is then converted into compound C by enzyme II. Compound C is the yellow pigment in the fruit. Plants with the genotype *ww* produce enzyme I and may be green or yellow, depending on whether enzyme II is present. When allele *Y* is present at a second locus, enzyme II is produced and compound B is converted into compound C, producing a yellow fruit. When two copies of allele *y*, which does not encode a functional form of enzyme II, are present, the fruit remains green. The presence of *W* at the first locus inhibits the conversion of compound A into compound B; plants with genotype *W_* do not make compound B, and their fruit remains white, regardless of which alleles are present at the second locus.



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5.9 Yellow pigment in summer squash is produced in a two-step biochemical pathway.

Description

Compound A is a colorless summer squash shown on the left side. An enzyme 1 with genotype small W small W converts compound A into compound B on the right side. A caption reads, 1: “Plants with genotype small W small W produce enzyme 1, which converts compound A (colorless) into compound B (green).” Another caption reads, 2: “Dominant allele big W inhibits the conversion of A into B.” Compound B is green in color. Another enzyme, called Enzyme 2 with genotype big Y blank coverts compound B into compound C on the extreme right side. Compound C is yellow in color. A caption reads, 3: “Plants with genotype big Y blank produce enzyme 2, which converts compound B into compound C (yellow).” Another caption reads, 4: “Plants with genotype small Y small Y do not encode a functional form of enzyme 2.”

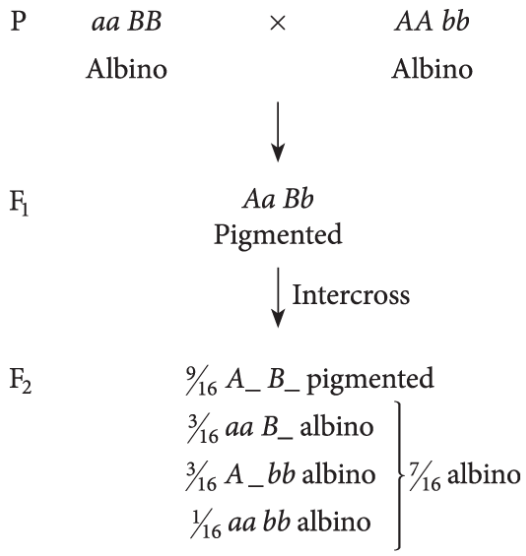
DUPLICATE RECESSIVE EPISTASIS

Finally, let’s consider duplicate recessive epistasis, in which two recessive alleles at either of two different loci are capable of suppressing a phenotype. This type of epistasis is illustrated by albinism in snails.

Albinism, the absence of pigment, is a common genetic trait in many plants and animals. Pigment is almost always produced through a multistep biochemical pathway; thus, albinism may entail gene interaction. Robert T. Dillon and Amy R. Wethington found that albinism in the common

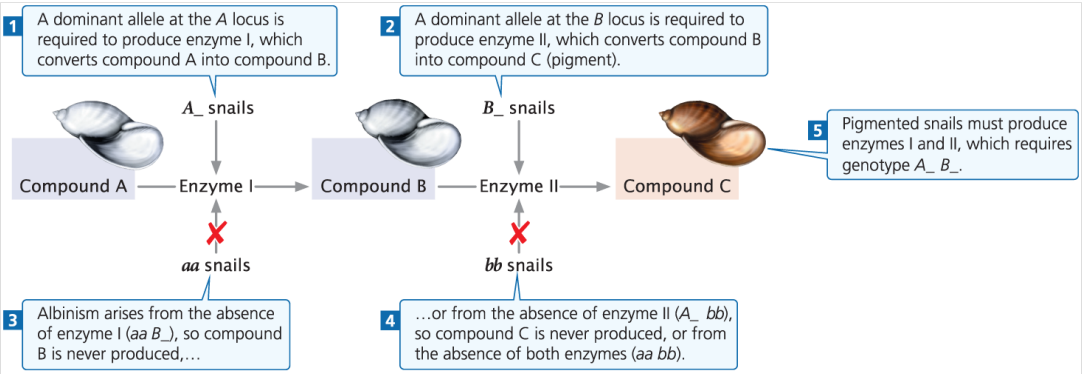
freshwater snail *Physa heterostropha* can result from the presence of two recessive alleles at either of two different loci. They collected inseminated snails from a natural population and placed them in cups of water, where the snails laid eggs. Some of the eggs hatched into albino snails. When two of these albino snails were crossed, all of the F_1 were pigmented. When the F_1 were intercrossed, the F_2 consisted of $^9/_{16}$ pigmented snails and $^7/_{16}$ albino snails. How did this 9:7 ratio arise?

The 9:7 ratio seen in the F_2 snails can be understood as a modification of the 9:3:3:1 ratio obtained when two individuals heterozygous for two loci are crossed. The 9:7 ratio arises when dominant alleles at both loci (*A*_ *B*_) produce pigmented snails and any other genotype produces albino snails:



Description
The P generation on the top shows two albinos: One albino has genotype small A small A big B big B and the other has genotype big A big A small B small B. They are crossed and a F subscript 1 generation is obtained below. The results of this cross include heterozygous progeny with genotype big A small A big B small B and are pigmented. Intercross produces the F subscript 2 generation below. The progeny are listed as follows: nine over sixteen big A blank big B blank, pigmented; three over sixteen small A small A big B blank, albino; three over sixteen big A blank small B small B, albino; and one over sixteen small A small A small B small B, albino. Thus, 7 over 16 in F subscript 2 generation are albino.

The 9:7 ratio in these snails can be explained by a two-step pathway of pigment production (Figure 5.10). Pigment (compound C) is produced only when compound A is converted into compound B by enzyme I and compound B is converted into compound C by enzyme II. At least one dominant allele *A* at the first locus is required to produce enzyme I; similarly, at least one dominant allele *B* at the second locus is required to produce enzyme II. Albinism arises from the absence of compound C, which may happen in one of three ways. First, two recessive alleles at the first locus (genotype *aa B_*) may prevent the production of enzyme I, so compound B is never produced. Second, two recessive alleles at the second locus (genotype *A_ bb*) may prevent the production of enzyme II; in this case, compound B is never converted into compound C. Third, two recessive alleles may be present at both loci (*aa bb*), causing the absence of both enzyme I and enzyme II. In this example of gene interaction, *a* is epistatic to *B*, and *b* is epistatic to *A*; *both* are recessive epistatic alleles because the presence of two copies of either allele *a* or allele *b* is necessary to suppress pigment production. This example differs from the suppression of coat color in Labrador retrievers in that recessive alleles at either of two loci are capable of suppressing pigment production in the snails, whereas recessive alleles at a single locus suppress pigment expression in the dogs.



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5.10 Pigment is produced in a two-step biochemical pathway in snails.

Description
Compound A on the left side is a colorless snail and an enzyme 1 with genotype big A blank converts it into compound B on the right side, which is also colorless. A caption reads, 1: “A dominant allele at the big A locus is required to produce enzyme 1, which converts compound A into compound B.” Another caption reads, 3: “Albinism arises from the absence of enzyme 1 (small A small A big B blank), so compound B is never produced ellipsis”. Another enzyme, called Enzyme 2 with genotype big B blank coverts compound B into compound C on the extreme right side, which is pigmented. A caption reads, 2: “A dominant allele at the big B locus is required to produce enzyme 2, which converts compound B into compound C (pigment).” Another caption reads, 4: “ellipsis or from the absence of enzyme 2 (big A blank small B small B), so compound C is never produced, or from the absence of both enzymes (small A small A small B small B).” The last caption reads, “Pigmented snails must produce enzymes 1 and 2, which requires genotype big A blank big B blank.”

CONCEPTS

Epistasis is the masking of the expression of one gene by another gene at a different locus. The epistatic gene does the masking; the hypostatic gene is masked. Epistatic alleles can be dominant or recessive.

✔ CONCEPT CHECK 7

A number of all-white cats are crossed, and they produce the following types of progeny: $^{12}/_{16}$ all-white, $^{3}/_{16}$ black, and $^{1}/_{16}$ gray. What is the genotype of the black progeny?

a. *Aa*
b. *Aa Bb*
c. *A_ B_*
d. *A_ bb*

CONNECTING CONCEPTS CONNECT

Interpreting Phenotypic Ratios Produced by Gene Interaction

A number of modified phenotypic ratios that result from gene interaction are shown in **Table 5.2**. Each of these examples represents a modification of the basic 9:3:3:1 dihybrid ratio. In interpreting the genetic basis of these modified ratios, we should keep several points in mind. First, the inheritance of the genes

producing these characteristics is no different from the inheritance of genes encoding simple genetic characteristics. Mendel's principles of segregation and independent assortment still apply; each individual organism possesses two alleles at each locus, which separate in meiosis, and genes at the different loci assort independently. The only difference is in how the *products* of the genotypes interact to produce the phenotype. Thus, we cannot consider the expression of genes at each locus separately; instead, we must take into consideration how the genes at different loci interact.

TABLE 5.2 Modified dihybrid phenotypic ratios due to gene interaction

	Genotype					
Ratio*	***A_ B_***	***A_ bb***	***aa B_***	***aa bb***	**Type of Interaction**	**Example Discussed in Chapter**
9 : 3 : 3 : 1	9	3	3	1	None	Seed shape and seed color in peas
9 : 3 : 4	9	3	4		Recessive epistasis	Coat color in Labrador retrievers
12 : 3 : 1	12		3	1	Dominant epistasis	Color in squash
9 : 7	9	7			Duplicate recessive epistasis	Albinism in snails
9 : 6 : 1	9	6		1	Duplicate interaction	—
15 : 1	15			1	Duplicate dominant epistasis	—
13 : 3	13		3		Dominant and recessive epistasis	—

*Each ratio is produced by a dihybrid cross (*Aa Bb* × *Aa Bb*). Shaded bars represent combinations of genotypes that give the same phenotype.

Description

The table shows four genotypes. 1, upper case A underscore big B underscore. 2, big A underscore small B small B. 3, small A small A big B underscore. 4, small A small A small B small B. Ratio of the four genotypes is 9 to 3 to 3 to 1, respectively. Type of interaction is none and the example discussed in chapter is seed shape and seed color in peas. Ratio is 9 to 3 to 4 (4 collectively for the last two genotypes that give the same phenotype). Type of interaction is recessive epistasis and the example discussed in chapter is coat color in Labrador retrievers. Ratio is 12 to 3 to 1 (12 collectively for the first two genotypes that give the same phenotype). Type of interaction is dominant epistasis and the example discussed in chapter is coat color in squash. Ratio is 9 to 7 (7 collectively for the last three genotypes that give the same phenotype). Type of interaction is duplicate recessive epistasis and example discussed in chapter is albinism in snails. Ratio is 9 to 6 to 1 (6 collectively for the second and third genotypes that give the same phenotype). Type of interaction is duplicate interaction and there is no example discussed in the chapter. Ratio is 15 to 1 (15 collectively for the first three genotypes that give the same phenotype). Type of interaction is duplicate dominant epistasis and there is no example discussed in the chapter. Ratio is 13 to 3 (13 collectively for the first, second, and fourth genotypes that give the same phenotype). Type of interaction is dominant and recessive epistasis and there is no example discussed in the chapter. A caption at the bottom of the table

reads each ratio is produced by a dihybrid cross (big A small A cross big A small A big B small B). Shaded bars represent combinations of genotypes that give the same phenotype.

A second point is that in the examples that we have considered, the phenotypic proportions were always in sixteenths because, in all the crosses, pairs of alleles segregated at two independently assorting loci. The probability of inheriting one of the two alleles at a locus is $^1/_2$. Because there are two loci, each with two alleles, the probability of inheriting any particular combination of genes is $(^1/_2)^4 = {}^1/_{16}$. For a trihybrid cross, the progeny proportions should be in sixty-fourths, because $(^1/_2)^6 = {}^1/_{64}$. In general, the progeny proportions should be in fractions of $(^1/_2)^{2n}$, where *n* equals the number of loci with two alleles segregating in the cross.

Crosses rarely produce exactly 16 progeny; therefore, modifications of the dihybrid ratio are not always obvious. Modified dihybrid ratios are more easily seen if the number of individuals of each phenotype is expressed in sixteenths:

$$\frac{x}{16} = \frac{\text{number of progeny with a phenotype}}{\text{total number of progeny}}$$

where *x*/16 equals the proportion of progeny with a particular phenotype. If we solve for *x* (the proportion of the particular phenotype in sixteenths), we have

$$x = \frac{\text{number of progeny with a phenotype} \times 16}{\text{total number of progeny}}$$

For example, suppose that we cross two homozygotes, interbreed the F_1, and obtain 63 red, 21 brown, and 28 white F_2 individuals. Using the preceding formula, we find the phenotypic ratio in the F_2 to be red = (63 × 16) / 112 = 9; brown = (21 × 16) / 112 = 3; and white = (28 × 16) / 112 = 4. The phenotypic ratio is 9:3:4.

A final point to consider is how to assign genotypes to the phenotypes in modified ratios that result from gene interaction. Don't try to *memorize* the genotypes associated with all the modified ratios in Table 5.2. Instead, practice relating modified ratios to known ratios, such as the 9:3:3:1 dihybrid ratio. Suppose that we obtain $^{15}/_{16}$ green progeny and $^1/_{16}$ white progeny in a cross between two plants. If we compare this 15:1 ratio with the standard 9:3:3:1 dihybrid ratio, we see that $^9/_{16} + {}^3/_{16} + {}^3/_{16}$ equals $^{15}/_{16}$. All the genotypes associated with these proportions in the dihybrid cross (*A_ B_*, *A_ bb*, and *aa B_*) must give the same phenotype, the green progeny. Genotype *aa bb* makes up $^1/_{16}$ of the progeny in a dihybrid cross—the white progeny in this cross.

In assigning genotypes to phenotypes in modified ratios, students sometimes become confused about which letters to assign to which phenotype. Suppose that we obtain the following phenotypic ratio: $^9/_{16}$ black: $^9/_{16}$ brown: $^4/_{16}$ white. Which genotype do we assign to the brown progeny, *A_ bb* or *aa B_*? Either answer is correct because the letters are just arbitrary symbols for the genetic information. The important

thing to realize about this ratio is that the brown phenotype arises when two recessive alleles are present at one locus.

WORKED PROBLEM

A homozygous strain of yellow corn is crossed with a homozygous strain of purple corn. The F_1 are intercrossed, producing an ear of corn with 119 purple kernels and 89 yellow kernels (the progeny). What is the genotype of the yellow kernels?

Solution Strategy

What information is required in your answer to the problem?

The genotype of the yellow kernels.

What information is provided to solve the problem?

- A homozygous yellow corn plant is crossed with a homozygous purple corn plant.
- The numbers of purple and yellow progeny produced by the cross.

Solution Steps

We should first consider whether the cross between yellow and purple strains might be a monohybrid cross for a simple dominant trait, which would produce a 3:1 ratio in the F_2 ($Aa \times Aa \rightarrow {}^3/_4 A_$ and ${}^1/_4\, aa$). Under this hypothesis, we would expect 156 purple progeny and 52 yellow progeny:

Phenotype	Genotype	Observed number	Expected number
purple	*A_*	119	$^3/_4 \times 208 = 156$
yellow	*aa*	89	$^1/_4 \times 208 = 52$
Total		208	

We see that the expected numbers do not closely fit the observed numbers. If we performed a chi-square test (see Chapter 3), we would obtain a calculated chi-square value of 35.08, which has a probability much less than 0.05, indicating that it is extremely unlikely that when we expect a 3:1 ratio, we would obtain 119 purple progeny and 89 yellow progeny just by chance. Therefore, we can reject the hypothesis that these results were produced by a monohybrid cross.

Another possible hypothesis is that the observed F_2 progeny are in a 1:1 ratio. However, we learned in Chapter 3 that a 1:1 ratio is produced by a cross between a heterozygote and a homozygote (*Aa* × *aa)*, and in this cross, both original parental strains were homozygous. Furthermore, a chi-square test comparing the observed numbers with an expected 1:1 ratio yields a calculated chi-square value of 4.32, which has a probability of less than 0.05.

Next, we should look to see if the results can be explained by a dihybrid cross (*Aa Bb* × *Aa Bb*). A dihybrid cross results in phenotypic proportions that are in sixteenths. We can apply the formula given earlier in the chapter to determine the number of sixteenths for each phenotype:

$$
\begin{aligned}
x &= \frac{\text{number of progeny with a phenotype} \times 16}{\text{total number of progeny}} \\
x_{(\text{purple})} &= \frac{119 \times 16}{208} = 9.15 \\
x_{(\text{yellow})} &= \frac{89 \times 16}{208} = 6.85
\end{aligned}
$$

Thus, purple and yellow appear in an approximate ratio of 9:7.

We can test this hypothesis with a chi-square test:

Phenotype	Genotype	Observed number	Expected number
purple	?	119	$^{9}/_{16} \times 208 = 117$
yellow	?	89	$^{7}/_{16} \times 208 = 91$
Total		208	

$$
\begin{aligned}
\chi^2 &= \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}} \\
&= \frac{(119 - 117)^2}{117} + \frac{(89 - 91)^2}{91} \\
&= 0.034 + 0.044 = 0.078 \\
\text{Degree of freedom} &= n - 1 = 2 - 1 = 1 \\
P &> 0.05
\end{aligned}
$$

The probability associated with the chi-square value is greater than 0.05, indicating that there is a good fit between the observed results and a 9:7 ratio.

We now need to determine how a dihybrid cross can produce a 9:7 ratio and what genotypes correspond to the two phenotypes. A dihybrid cross without epistasis produces a 9:3:3:1 ratio:

$$Aa\ Bb \times Aa\ Bb$$

$$\downarrow$$

$$A_\ B_\ {}^{9}/_{16}$$
$$A_\ bb\ {}^{3}/_{16}$$
$$aa\ B_\ {}^{3}/_{16}$$
$$aa\ bb\ {}^{1}/_{16}$$

Description
The illustration shows big A small A big B small B crossed with big A small A big B small B. An arrow pointing downward points to the 4 different types of progeny as follows: big A blank big B blank describes nine over sixteen of the offspring. Big A blank small B small B are three over sixteen of the progeny. Small A small A big B blank are three over sixteen of the offspring. Small A small A small B small B are one over sixteen of the offspring.

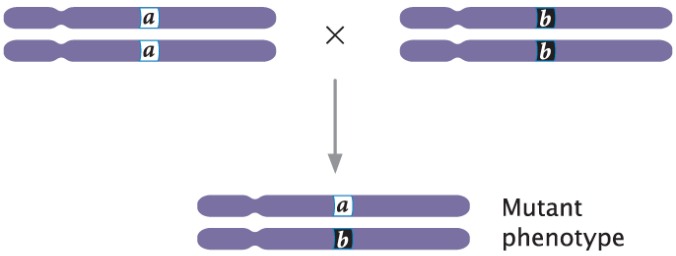
Because ${}^{9}/_{16}$ of the progeny from the corn cross are purple, purple must be produced by genotypes *A*_ *B*_; in other words, individual kernels that have at least one dominant allele at the first locus and at least one dominant allele at the second locus are purple. The proportions of all the other genotypes (*A*_ *bb*, *aa B*_, and *aa bb*) sum to ${}^{7}/_{16}$, which is the proportion of the progeny in the corn cross that are yellow, so any individual kernel that does not have a dominant allele at both the first and the second locus is yellow.

» Now test your understanding of epistasis by working **Problem 26** at the end of the chapter.

Complementation: Determining Whether Mutations Are at the Same Locus or at Different Loci

How do we know whether different mutations that affect a characteristic occur at the same locus (are allelic) or at different loci? In fruit flies, for example, *white* is an X-linked recessive mutation that produces white eyes instead of the red eyes found in wild-type flies; *apricot* is an X-linked recessive mutation that produces light orange eyes. Do the *white and apricot* mutations occur at the same locus or at different loci? We can use the complementation test to answer this question.

To carry out a **complementation test** on recessive mutations (*a* and *b*), parents that are homozygous for different mutations are crossed, producing offspring that are heterozygous. If the mutations are allelic (occur at the same locus), then the heterozygous offspring have only mutant alleles (*a b*) and exhibit a mutant phenotype:



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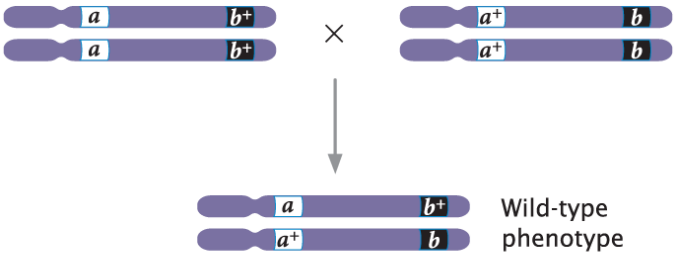
Description
The first homologous pair has small A written at one locus on the long arm of both of its chromosomes. The second homologous pair has small B written at one locus on the long arm of both of its chromosomes. An arrow pointing downwards shows the resultant homologous chromosome with a mutant phenotype with small A written on one chromosome at a locus and small B on another.

If, on the other hand, the mutations occur at different loci, each of the homozygous parents possesses wild-type genes at the other locus (*aa* b^+b^+ and a^+a^+ *bb*); so the heterozygous offspring inherit a mutant allele and a wild-type allele at each locus. In this case, the presence of a wild-type allele complements the mutation at each locus, and the heterozygous offspring have the wild-type phenotype:



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Description
The first homologous pair has small A written at one locus and small B superscript positive written at another locus on the long arm of both of its chromosomes. The second homologous pair has small A superscript positive written at one locus and small B written at another locus on the long arm of both of its chromosomes. An arrow pointing downward shows the resultant homologous chromosome with a wild-type phenotype with small A at a locus and small B superscript positive at another locus on one chromosome and small A superscript positive at a locus and small B at another locus on the other chromosome.

Complementation has taken place if an individual organism possessing two recessive mutations has a wild-type phenotype, indicating that the mutations are at nonallelic genes. There is a lack of complementation when two recessive mutations occur at the same locus, producing a mutant phenotype.

When the complementation test is applied to *white* and *apricot* mutations, all the heterozygous offspring have light-colored eyes, demonstrating that white eyes and apricot eyes are produced by mutations that occur at the same locus and are allelic.

THINK-PAIR-SHARE Question 2

CONCEPTS

A complementation test is used to determine whether two mutations occur at the same locus (are allelic) or at different loci.

✔ CONCEPT CHECK 8

Brindle (tiger-striped appearance) is a recessive trait in bulldogs and in Chihuahuas. What types of crosses would you carry out to determine whether the *brindle* genes in bulldogs and in Chihuahuas are at the same locus?

The Complex Genetics of Coat Color in Dogs

The genetics of coat color in dogs is an excellent example of the complex interactions between genes that may take part in the determination of a phenotype. Domestic dogs come in an amazing variety of shapes, sizes, and colors. For thousands of years, people have been breeding dogs for particular traits, producing the large number of types that we see today. Each breed of dog carries a selection of alleles from the ancestral dog gene pool; these alleles define the features of a particular breed. The genome of the domestic dog was completely sequenced in 2004, greatly facilitating the study of canine genetics.

Here we consider four loci (in the list that follows) that are important in producing many of the noticeable differences in color and pattern among breeds of dogs. In interpreting the genetic basis of differences in the coat colors of dogs, consider how the expression of a particular gene is modified

by the effects of other genes. Keep in mind that additional loci not listed here can modify the colors produced by these four loci and that not all geneticists agree on the genetics of color variation in some breeds.

1. **Agouti (*A*) locus.** This locus has five common alleles that determine the depth and distribution of color in a dog's coat:

A^s	Solid black pigment.
a^w	Agouti, or wolflike gray. Hairs encoded by this allele have a salt-and-pepper appearance, produced by a band of yellow pigment on a black hair.
a^y	Yellow. The black pigment is markedly reduced, so the entire hair is yellow.
a^s	Saddle markings (dark color on the back, with extensive tan markings on the head and legs).
a^t	Bicolor (dark color over most of the body, with tan markings on the feet and eyebrows).

   Alleles A^s and a^y are generally dominant to the other alleles, but the dominance relations are complex and not yet completely understood.
2. **Black (*B*) locus.** This locus determines whether black pigment can be formed. The actual color of a dog's coat depends on the effects of genes at other loci (such as the *A* and *E* loci). Two alleles are common:

B	Allows black pigment to be produced.
b	Black pigment cannot be produced; pigmented dogs can be

	chocolate, liver, tan, or red.

Allele *B* is dominant to allele *b*.

3. **Extension (*E*) locus.** Four alleles at this locus determine where the genotype at the *A* locus is expressed. For example, if a dog has the A^s allele (solid black) at the *A* locus, then black pigment will either be extended throughout the coat or be restricted to some areas, depending on the alleles present at the *E* locus. Areas where the *A* locus is not expressed may appear yellow, red, or tan, depending on the presence of particular alleles at other loci. When A^s is present at the *A* locus, the four alleles at the *E* locus have the following effects:

E^m	Black mask with a tan coat.
E	The *A* locus is expressed throughout (solid black).
e^{br}	Brindle, in which black and yellow are in layers to give a tiger-striped appearance.
e	No black in the coat, but the nose and eyes may be black.

The dominance relations among these alleles are poorly known.

4. **Spotting (*S*) locus**. Alleles at this locus determine whether white spots will be present. There are four common alleles:

S	No spots.
s^i	Irish spotting: numerous white spots.
s^p	Piebald spotting: various amounts of white.

s^w	Extreme white piebald: almost all white.

Allele *S* is completely dominant to alleles s^i, s^p, and s^w; alleles s^i and s^p are dominant to allele s^w ($S > s^i$, $s^p > s^w$). The relation between s^i and s^p is poorly defined; indeed, they may not be separate alleles. Genes at other poorly known loci also modify spotting patterns.

To illustrate how genes at these loci interact in determining a dog's coat color, let's consider a few examples.

LABRADOR RETRIEVER

Labrador retrievers (Figure 5.11a) may be black, brown, or yellow. Most are homozygous A^sA^s *SS*; thus, they vary only at the *B* and *E* loci. The A^s allele allows dark pigment to be expressed; whether a dog is black depends on which genes are present at the *B* and *E* loci. As discussed earlier in the chapter, all black Labradors must carry at least one *B* allele and one *E* allele (*B*_ *E*_). Brown dogs are homozygous *bb* and have at least one *E* allele (*bb E*_). Yellow dogs are a result of the presence of *ee* (*B*_ *ee* or *bb ee*). Labrador retrievers are homozygous for the *S* allele, which produces a solid color; the few white spots that appear in some dogs of this breed are due to other modifying genes.

Part a: imagebroker/Alamy. Part b: RFcompany/age fotostock. Part c: Stockbyte/Getty Images.

5.11 Coat color in dogs is determined by interactions between genes at a number of loci. (a) Most Labrador retrievers are genotype A^SA^S *SS*, varying only at the *B* and *E* loci. (b) Most

beagles are genotype $a^S a^S$ *BB* $s^P s^P$. (c) Dalmatians are genotype $A^S A^S$ *EE* $s^w s^w$, varying at the *B* locus, which makes the dogs black (*B_*) or brown (*bb*).

BEAGLE

Most beagles (Figure 5.11b) are homozygous $a^s a^s$ *BB* $s^p s^p$, although other alleles at these loci are occasionally present. The a^s allele produces the saddle markings—dark back and sides, with tan head and legs—that are characteristic of the breed. Allele *B* allows black to be produced, but its distribution is limited by the a^s allele. Most beagles are *E_*, but the genotype *ee* does occasionally arise, leading to a few all-tan beagles. White spotting in beagles is due to the s^P allele.

DALMATIAN

Dalmatians (Figure 5.11c) have an interesting genetic makeup. Most are homozygous $A^s A^s$ *EE* $s^w s^w$, so they vary only at the *B* locus. Notice that these dogs possess genotype $A^s A^s$ *EE*, which allows for a solid coat that would be black if genotype *B_* were present, or brown (called liver) if genotype *bb* were present. However, the presence of the s^w allele produces a white coat, masking the expression of the solid color. The dog's color appears only in the pigmented spots, which are due to the presence of an allele at yet another locus that allows the color to penetrate in a limited number of spots.

Table 5.3 gives the common genotypes of other breeds of dogs. **› TRY PROBLEM 33**

TABLE 5.3 Common genotypes in different breeds of dogs

Breed	Usual Homozygous Genotypes*	Other Alleles Present Within the Breed

Basset hound	*BB EE*	a^{y}, a^{t} *S*, s^{P}, s^{i}
Beagle	$a^{s}a^{s}$ *BB* $s^{P}s^{P}$	*E*, *e*
Chihuahua		A^{s}, a^{y}, a^{s}, a^{t} *B*, *b* E^{m}, *E*, e^{br}, *e S*, s^{i}, s^{P}, s^{w}
Collie	*BB EE*	a^{y}, a^{t}, s^{i}, s^{w}
Dalmatian	$A^{s}A^{s}$ *EE* $s^{w}s^{w}$	*B*, *b*
Doberman	$a^{t}a^{t}$ *EE SS*	*B*, *b*
English bulldog	*BB*	A^{s}, a^{y}, a^{t} E^{m}, *E*, e^{br} *S*, s^{i}, s^{P}, s^{w}
German shepherd	*BB SS*	a^{y}, *a*, a^{s}, a^{t} E^{m}, *E*, *e*
Golden retriever	$A^{s}A^{s}$ *BB SS*	*E*, *e*
Greyhound	*BB*	A^{s}, a^{y} *E*, e^{br}, *e S*, s^{P}, s^{w} s^{i}
Irish setter	*BB ee SS*	A^{s}, a^{t}
Labrador retriever	$A^{s}A^{s}$ *SS*	*B*, *b E*, *e*
Poodle	*SS*	A^{s}, a^{t} *B*, *b E*, *e*
Rottweiler	$a^{t}a^{t}$ *BB EE SS*	
St. Bernard	$a^{y}a^{y}$ *BB*	E^{m}, *E* s^{i}, s^{P}, s^{w}

* Most dogs in the breed are homozygous for these genes; a few individual dogs may possess other alleles at these loci. Data from: M. B. Willis, *Genetics of the Dog* (London: Witherby, 1989).

5.3 Sex Influences the Inheritance and Expression of Genes in a Variety of Ways

In Chapter 4, we considered characteristics encoded by genes located on the sex chromosomes (sex-linked traits) and how their inheritance differs from the inheritance of characteristics encoded by autosomal genes. X-linked traits, for example, are passed from father to daughter but never from father to son, and Y-linked traits are passed from father to all sons. Here we examine additional influences of sex, including the effect of the sex of an individual organism on the expression of autosomal genes, on characteristics determined by genes located in the cytoplasm, and on characteristics for which the genotype of only the maternal parent determines the phenotype of the offspring. Finally, we look at situations in which the expression of autosomal genes is affected by the sex of the parent from whom the genes are inherited.

Sex-Influenced and Sex-Limited Characteristics

Sex-influenced characteristics are determined by autosomal genes and are inherited according to Mendel's principles, but they are expressed differently in males and females. In this case, a particular

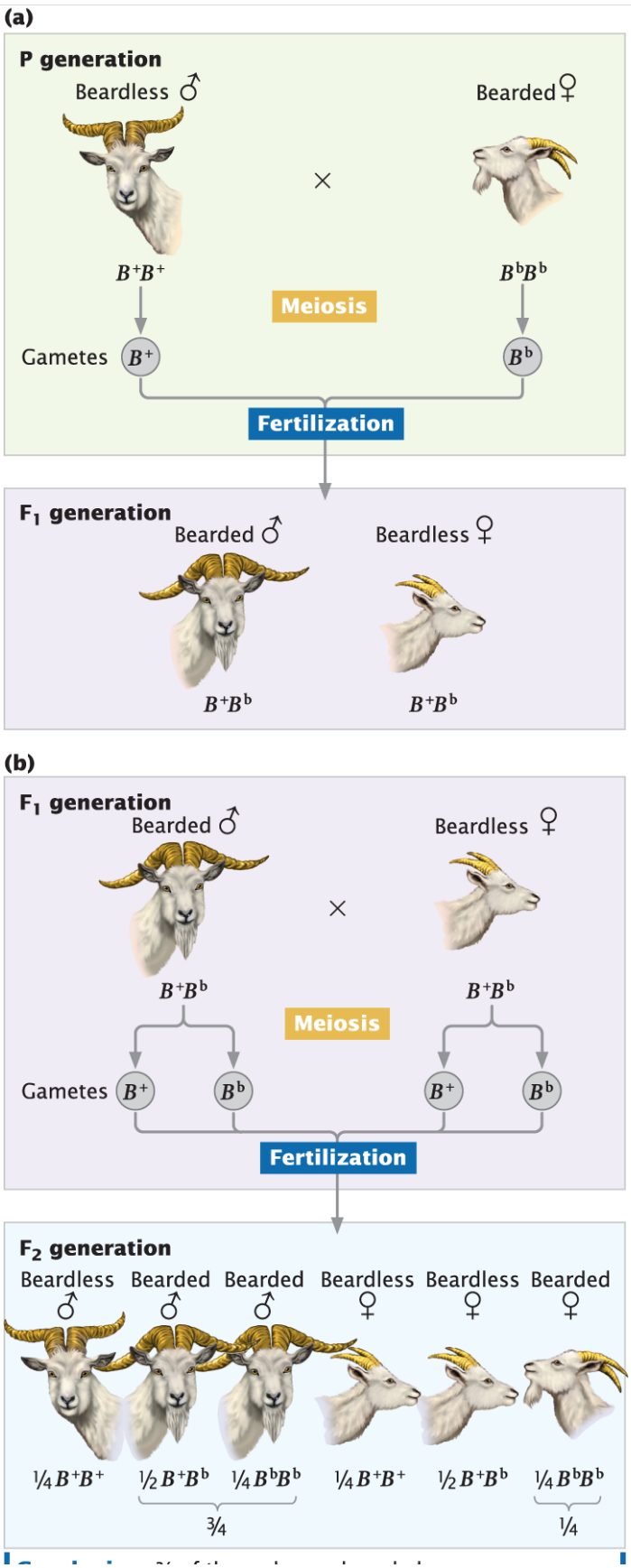
trait is more readily expressed in one sex; in other words, the trait has higher penetrance in one of the sexes.

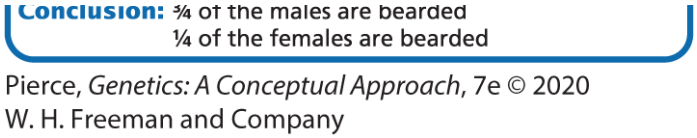
For example, the presence of a beard on some goats is determined by an allele at an autosomal locus (B^b) that is dominant in males and recessive in females. In males, a single beard allele is required for the expression of this trait: both the homozygote ($B^b\ B^b$) and the heterozygote ($B^b\ B^+$) have beards, whereas the $B^+\ B^+$ male is beardless. In contrast, females require two beard alleles in order for this trait to be expressed: the homozygote $B^b\ B^b$ has a beard, whereas the heterozygote ($B^b\ B^+$) and the other homozygote ($B^+\ B^+$) are beardless.

Genotype	Males	Females
$B^+\ B^+$	beardless	beardless
$B^+\ B^b$	bearded	beardless
$B^b\ B^b$	bearded	bearded

The key to understanding the expression of the beard allele is to look at the heterozygote. In males (for which the presence of a beard is dominant), the heterozygous genotype produces a beard, but in females (for which the absence of a beard is dominant), the heterozygous genotype produces a goat without a beard.

Figure 5.12a illustrates a cross between a beardless male ($B^+ B^+$) and a bearded female ($B^b B^b$). The alleles separate into gametes according to Mendel's principle of segregation, and all the F_1 are heterozygous ($B^+ B^b$). Because the trait is dominant in males and recessive in females, all the F_1 males will be bearded and all the F_1 females will be beardless. When the F_1 are crossed with each other, $^1/_4$ of the F_2 progeny are $B^b B^b$, $^1/_2$ are $B^b B^+$, and $^1/_4$ are $B^+ B^+$ (**Figure 5.12b**). Because male heterozygotes are bearded, $^3/_4$ of the males in the F_2 possess beards; because female heterozygotes are beardless, only $^1/_4$ of the females in the F_2 are bearded.


(a)
P generation
Beardless ♂
Bearded ♀
×
B^+B^+
B^bB^b
Meiosis
Gametes
B^+
B^b
Fertilization
F1 generation
Bearded ♂
Beardless ♀
B^+B^b
B^+B^b
(b)
F1 generation
Bearded ♂
Beardless ♀
×
B^+B^b
B^+B^b
Meiosis
Gametes
B^+
B^b
B^+
B^b
Fertilization
F2 generation
Beardless
Bearded
Bearded
Beardless
Beardless
Bearded
♂
♂
♂
♀
♀
♀
¼ B^+B^+
½ B^+B^b
¼ B^bB^b
¼ B^+B^+
½ B^+B^b
¼ B^bB^b
¾
¼



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5.12 Genes that encode sex-influenced characteristics are inherited according to Mendel's principles but are expressed differently in males and females.

Description

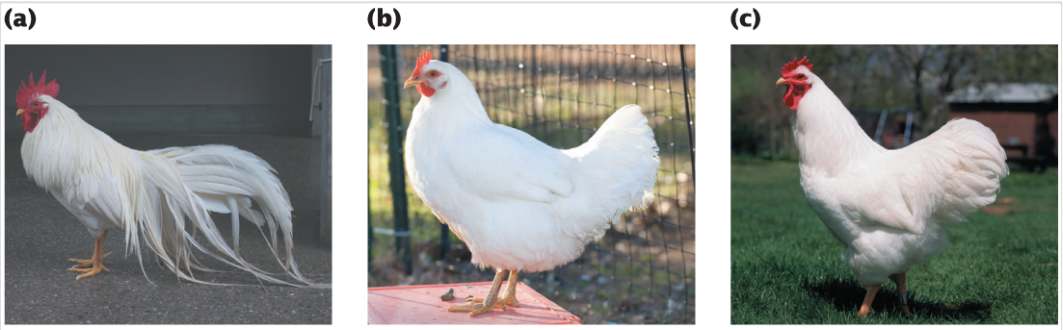
The part (a) on the top shows a cross between a beardless male goat with genotype big B superscript positive big B superscript positive and a bearded female goat with genotype big B superscript small B big B superscript small B in the P generation. After meiosis, the male goat produces big B superscript positive gametes and the female goat produces big B superscript small B gametes. Fertilization occurs and leads to F subscript 1 generation below which consists of a bearded male with genotype big B superscript positive big B superscript small B and a beardless female with genotype big B superscript positive big B superscript small B.

The part (b) below shows a cross in the F subscript 1 generation to produce F subscript 2 generation. The progeny produced in F subscript 1 generation are crossed. Meiosis results in the production of big B superscript positive and big B superscript small B gametes from each of the parents. These gametes undergo fertilization and produces F subscript 2 generation below as follows: one-fourth beardless male with genotype big B superscript positive big B superscript positive; one-half bearded males with genotype big B superscript positive big B superscript small B; one-fourth bearded males with genotype big B superscript small B big B superscript small B; one-fourth beardless females with genotype big B superscript positive big B superscript positive; one-half beardless females with genotype big B superscript positive big B superscript small B; one-fourth bearded females with genotype big B superscript small B big B superscript small B.

A caption at the bottom reads, "Conclusion: three-fourth of the males are bearded, one fourth of the females are bearded."

A **sex-limited characteristic** is encoded by autosomal genes that are expressed in only one sex; the trait has zero penetrance in the other sex. In domestic chickens, for example, some males display a

plumage pattern called cock feathering (Figure 5.13a). Other males and all females display a pattern called hen feathering (Figure 5.13b and c). Cock feathering is an autosomal recessive trait that is limited to males. Because the trait is autosomal, the same genotypes are found in males and females, but the phenotypes produced by these genotypes differ between males and females:



Part a: Moment Open/Getty Images. Part b: Guy J. Sagi/Shutterstock. Part c: James Marshall/Getty Images.

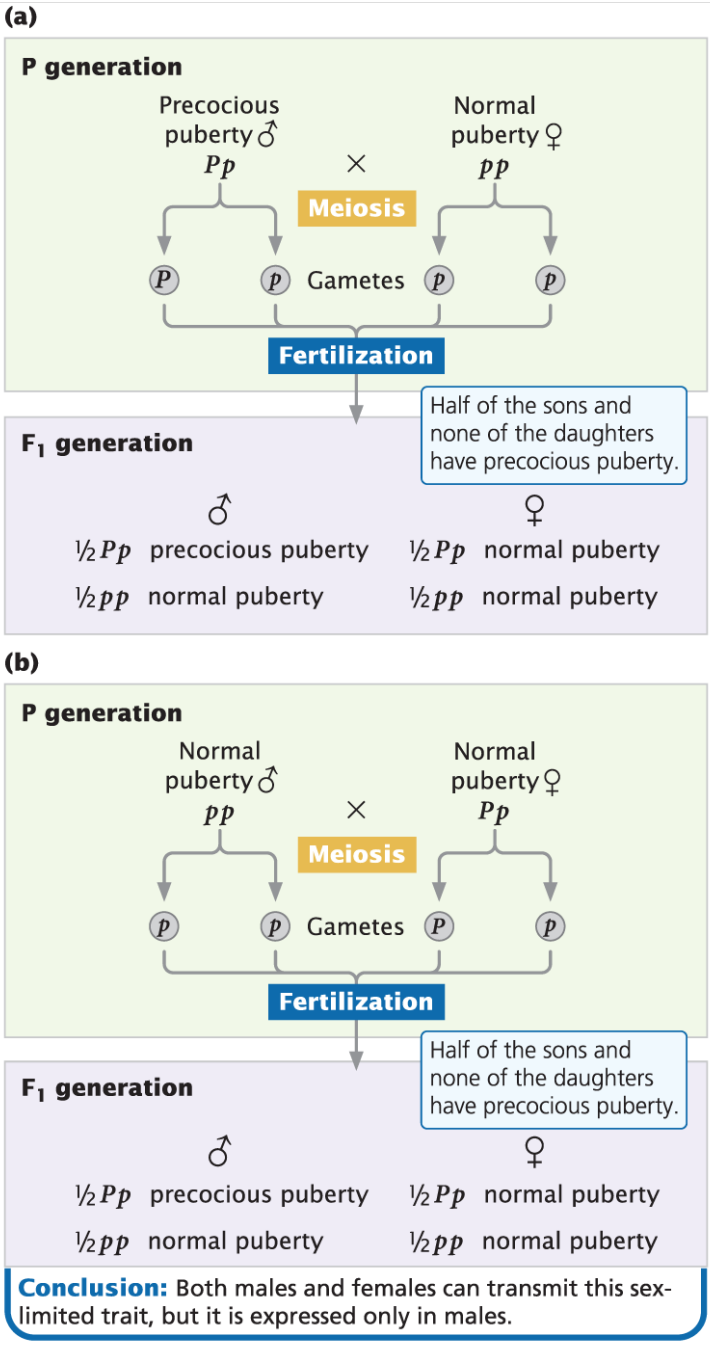
5.13 A sex-limited characteristic is encoded by autosomal genes that are expressed in only one sex. An example is cock feathering in chickens, an autosomal recessive trait that is limited to males. (a) Cock-feathered male. (b) Hen-feathered female. (c) Hen-feathered male.

Genotype	Male phenotype	Female phenotype
HH	hen feathering	hen feathering
Hh	hen feathering	hen feathering
hh	cock feathering	hen feathering

An example of a sex-limited characteristic in humans is male-limited precocious puberty. There are several types of precocious puberty in humans, most of which are not genetic. Male-limited precocious puberty, however, results from an autosomal dominant allele (*P*) that is expressed only in males; females with this allele are normal in phenotype. Males with precocious puberty undergo puberty at an early age, usually before the age of four. At this time, the penis enlarges, the voice deepens, and pubic hair develops. There is no impairment of sexual function; affected males are fully fertile. Most are short as adults because the long bones stop growing after puberty.

Because the trait is rare, affected males are usually heterozygous (*Pp*). A male with precocious puberty who mates with a woman who has no family history of this condition will transmit the allele for precocious puberty to half of their children (**Figure 5.14a**), but it will be expressed only in the sons. If one of the heterozygous daughters (*Pp*) mates with a male who exhibits normal puberty (*pp*), half of their sons will exhibit precocious puberty (**Figure 5.14b**). Thus, a sex-limited characteristic can be inherited from either parent, although the trait appears in only one sex. **› TRY PROBLEM 35**

THINK-PAIR-SHARE Questions 3, 4, and 5



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5.14 Sex-limited characteristics are inherited according to Mendel's principles.

Precocious puberty is an autosomal dominant trait that is limited to males.

Description

Part (a) shows a cross between a male with precocious puberty with genotype big P small P and a normal puberty female with genotype small P small P. Meiosis occurs and results in the formation of gametes below. Big P and small P are the gametes formed from male while small P and small P are formed from female. Fertilization occurs and results in the formation of F subscript 1 generation below. A caption reads, “Half of the sons and none of the daughters have precocious puberty.” F subscript 1 generation are as follows: one-half precocious puberty males of genotype big P small P; one-half normal puberty males of genotype small P small P; one-half normal puberty females of genotype big P small P; and one-half of normal puberty females of genotype small P small P.

Part (b) shows a cross between a male with normal puberty with genotype small P small P and a normal puberty female with genotype big P small P. Meiosis occurs and results in the formation of gametes below. Small P and small P are the gametes formed from male while big P and small P are formed from female. Fertilization occurs and results in the formation of F subscript 1 generation below. A caption reads, “Half of the sons and none of the daughters have precocious puberty.” F subscript 1 generation are as follows: one-half precocious puberty males of genotype big P small P; one-half normal puberty males of genotype small P small P; one-half normal puberty females of genotype big P small P; and one-half of normal puberty females of genotype small P small P. A caption below reads, “Conclusion: Both males and females can transmit this sex limited trait, but it is expressed only in males.”

CONCEPTS

Sex-influenced characteristics are encoded by autosomal genes that are more readily expressed in one sex. Sex-limited characteristics are encoded by autosomal genes whose expression is limited to one sex.

✔ CONCEPT CHECK 9

How do sex-influenced and sex-limited characteristics differ from sex-linked characteristics?

Cytoplasmic Inheritance

Mendel's principles of segregation and independent assortment are based on the assumption that genes are located on chromosomes in the nucleus of the cell. For most genetic characteristics, this assumption is valid, and Mendel's principles allow us to predict the types of offspring that will be produced in a genetic cross. Not all the genetic material of a cell is found in the nucleus, however. Some characteristics are encoded by genes located in the cytoplasm, and these characteristics exhibit **cytoplasmic inheritance**.

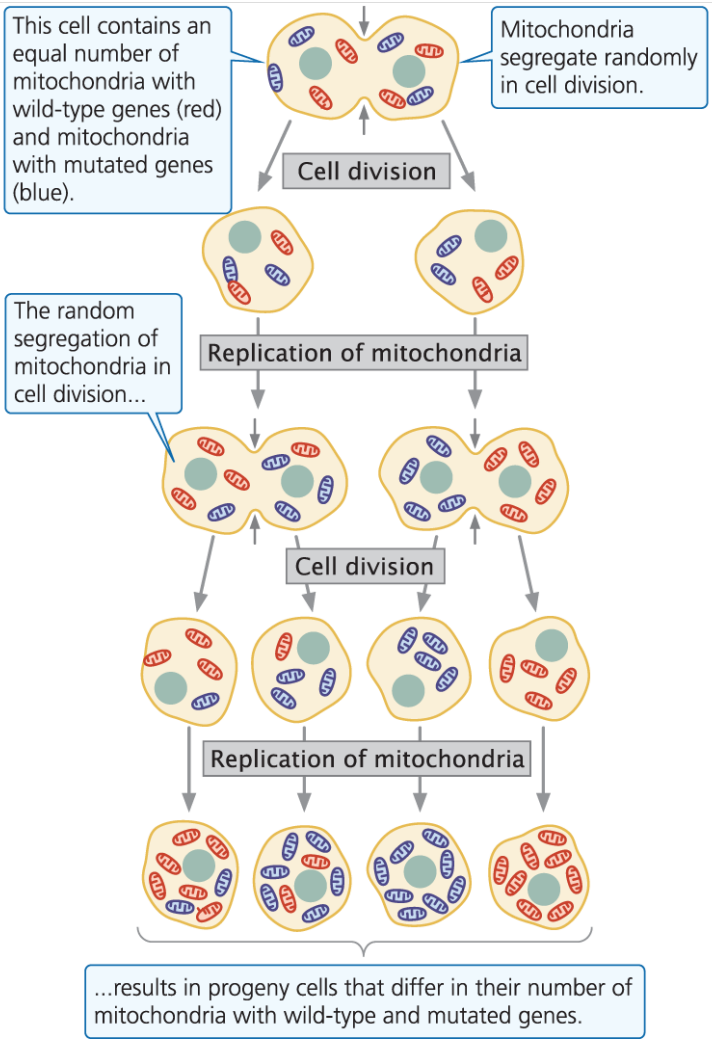
A few organelles, notably chloroplasts and mitochondria, contain DNA. The human mitochondrial genome contains about 16,569 nucleotides of DNA, encoding 37 genes. Compared with the nuclear genome, which contains some 3 billion nucleotides encoding some 20,000 genes, the mitochondrial genome is very small; nevertheless, mitochondrial and chloroplast genes encode some important characteristics. The molecular characteristics of this extranuclear DNA are discussed in Chapter 11; here, we focus on *patterns* of cytoplasmic inheritance.

Cytoplasmic inheritance differs from the inheritance of characteristics encoded by nuclear genes in several important respects. A zygote inherits nuclear genes from both parents, but typically all its cytoplasmic organelles, and thus all its cytoplasmic genes, come from only one of the gametes, usually the egg. A sperm from the male parent generally contributes only a set of nuclear

genes. Thus, most cytoplasmically inherited traits are present in both males and females and are passed from mother to offspring, never from father to offspring. Reciprocal crosses, therefore, give different results when cytoplasmic genes encode a trait. In a few organisms, however, cytoplasmic genes are inherited from the male parent only or from both parents.

Cytoplasmically inherited characteristics frequently exhibit extensive phenotypic variation because no mechanism analogous to mitosis or meiosis ensures that cytoplasmic genes are evenly distributed during cell division. Thus, different cells and different individual offspring will contain various proportions of cytoplasmic genes.

Consider mitochondrial genes. Most cells contain thousands of mitochondria, and each mitochondrion contains from 2 to 10 copies of the mitochondrial DNA (mtDNA). Suppose that half of the mitochondria in a cell contain a normal wild-type copy of mtDNA and the other half contain a mutated copy (**Figure 5.15**). During cell division, the mitochondria segregate into the progeny cells at random. Just by chance, one cell may receive mostly mutated mtDNA and another cell may receive mostly wild-type mtDNA. Therefore, different progeny from the same mother, and even different cells within an individual offspring, may vary in their phenotypes. Traits encoded by chloroplast DNA (cpDNA) are similarly variable. The characteristics exhibited by cytoplasmically inherited traits are summarized in **Table 5.4**.



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5.15 Cytoplasmically inherited characteristics frequently exhibit extensive phenotypic variation because different cells and different individual offspring may contain different proportions of cytoplasmic genes.

Description

The flowchart starts with a dividing parent cell with a constriction with one nucleus and four mitochondria (two blue and two red) on each side of the constriction. The first caption reads, “This cell contains an equal number of mitochondria with wild-type genes (in red) and mitochondria with mutated genes (in blue).” The second caption reads, “Mitochondria segregate randomly in cell division". First cell division takes place and daughter cells with equal number of red and blue mitochondria are produced below. Replication of

mitochondria occurs and two dividing cells similar to the initial parent cell are present. In the first cell, 3 red and 1 blue mitochondria are present on one side of constriction and 3 red and 1 blue are present on the other. In the second cell, 4 blue mitochondria are present on one side of the constriction and 4 red are present on the other. A caption reads, "The random segregation of

mitochondria in cell division ellipsis". Second cell division takes place and 4 daughter cells are produced below with different number of mitochondria. Cell 1 has 3 red and 1 blue, cell 2 has 1 red and 3 blue, cell 3 has 4 blue, and cell 4 has 4 red mitochondria. Replication of mitochondria takes place again and the 4 cells have doubled their number of mitochondria in the same color. A caption below reads, "ellipsis results in progeny cells that differ in their number of mitochondria with wild-type and mutated genes."

TABLE 5.4 Characteristics of cytoplasmically inherited traits

1. Present in males and females.
2. Usually inherited from one parent, typically the maternal parent.
3. Reciprocal crosses give different results.
4. Exhibit extensive phenotypic variation, even within a single family.

VARIEGATION IN FOUR-O'CLOCKS

In 1909, cytoplasmic inheritance was recognized by Carl Correns as an exception to Mendel's principles. Correns, one of the biologists

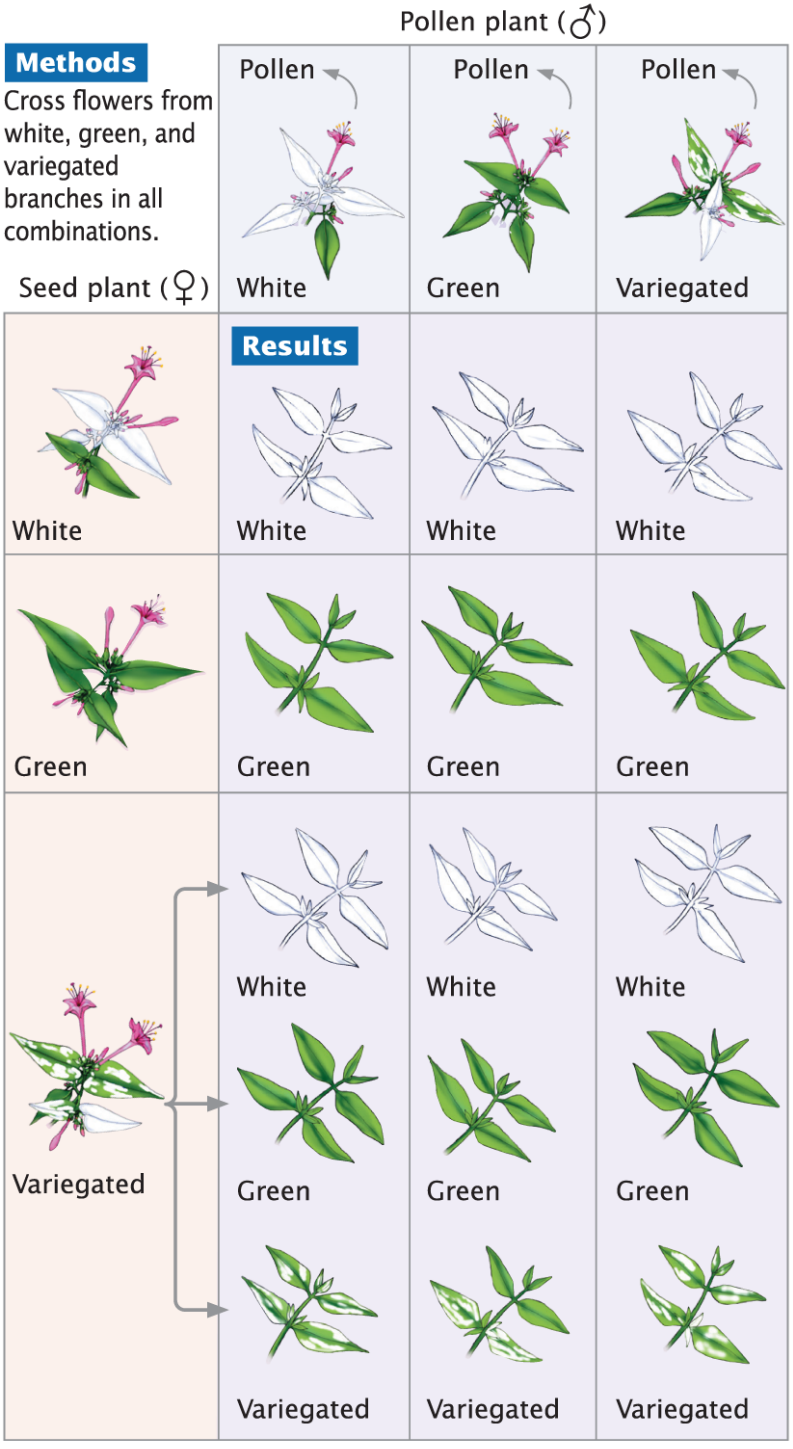
who rediscovered Mendel's work, studied the inheritance of leaf variegation in the four-o'clock plant, *Mirabilis jalapa*. Correns found that some of the leaves and stems of one variety of four-o'clock were variegated, displaying a mixture of green and white splotches. He also noted that some branches of the variegated strain had all-green leaves; other branches had all-white leaves. Each branch produced flowers, so Correns was able to cross flowers from variegated, green, and white branches in all combinations (**Figure 5.16**). The seeds from flowers on green branches always gave rise to green progeny, no matter whether the pollen was from a green, white, or variegated branch. Similarly, flowers on white branches always produced white progeny. Flowers on the variegated branches gave rise to green, white, and variegated progeny in no particular ratio.

Experiment

Question: How is stem and leaf color inherited in the four-o'clock plant?

Methods

Cross flowers from white, green, and variegated branches in all combinations.



Conclusion: The phenotype of the progeny is determined by the phenotype of the branch from which the seed originated, not from the branch on which the pollen originated. Stem

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5.16 Crosses for stem and leaf color in four-o'clocks illustrate cytoplasmic inheritance.

Description

The illustration begins with a question that reads, How is stem and leaf color inherited in the

four-o'clock plant? The methods show a text that reads, Cross fowers from white, green, and variegated branches in all

combinations. The table consists of 4 columns and 4 rows. In each cell of the table, is a picture of the plant and a word describing the phenotype. The first row and the first column include row and column headers respectively. The inner columns and rows (3 multiplied by 3) show the results of the crosses between the entries of each row header and column header. A text in the first column of the first row reads: Cross ¬flowers from white, green, and variegated branches in all combinations (methods). The column headers show leaf and stem colors of male pollen plant as follows: white, green, and variegated. The row headers show leaf and stem colors of female seed plant as follows: white, green, and variegated. The row entries of results for each column are as follows:

Row 1: White, white, white

Row 2: Green, green, green

For variegated, the result is branched into three parts for each column as follows:

Row 3: White, green, variegated; white, green, variegated; and white, green, variegated;

A caption at the bottom reads, "Conclusion: The phenotype of the progeny is determined by the phenotype of the branch from which the seed originated, not from the branch on which the pollen originated. Stem and leaf color exhibit cytoplasmic inheritance."

Correns's crosses demonstrated the cytoplasmic inheritance of variegation in four-o'clocks. The phenotypes of the offspring were determined entirely by the maternal parent, never by the paternal parent (the source of the pollen). Furthermore, the production of all three phenotypes by flowers on variegated branches is consistent with cytoplasmic inheritance. The white color in these plants is caused by a defective gene in cpDNA, which results in a failure to produce the green pigment chlorophyll. Cells from green branches contain normal chloroplasts only, cells from white branches contain abnormal chloroplasts only, and cells from variegated branches contain a mixture of normal and abnormal chloroplasts. In the flowers from variegated branches, the random segregation of chloroplasts in the course of oogenesis produces some egg cells with only normal cpDNA, which develop into green progeny; other egg cells with only abnormal cpDNA, which develop into white progeny; and still other egg cells with a mixture of normal and abnormal cpDNA, which develop into variegated progeny.

MITOCHONDRIAL DISEASES

A number of human diseases (mostly rare ones) that exhibit cytoplasmic inheritance have been identified. These disorders arise from mutations in mtDNA, most of which occur in genes encoding components of the electron-transport chain, which generates most of the ATP (adenosine triphosphate) in aerobic cellular respiration. One such disease is Leber hereditary optic neuropathy (LHON). People who have this disorder experience rapid loss of vision in both

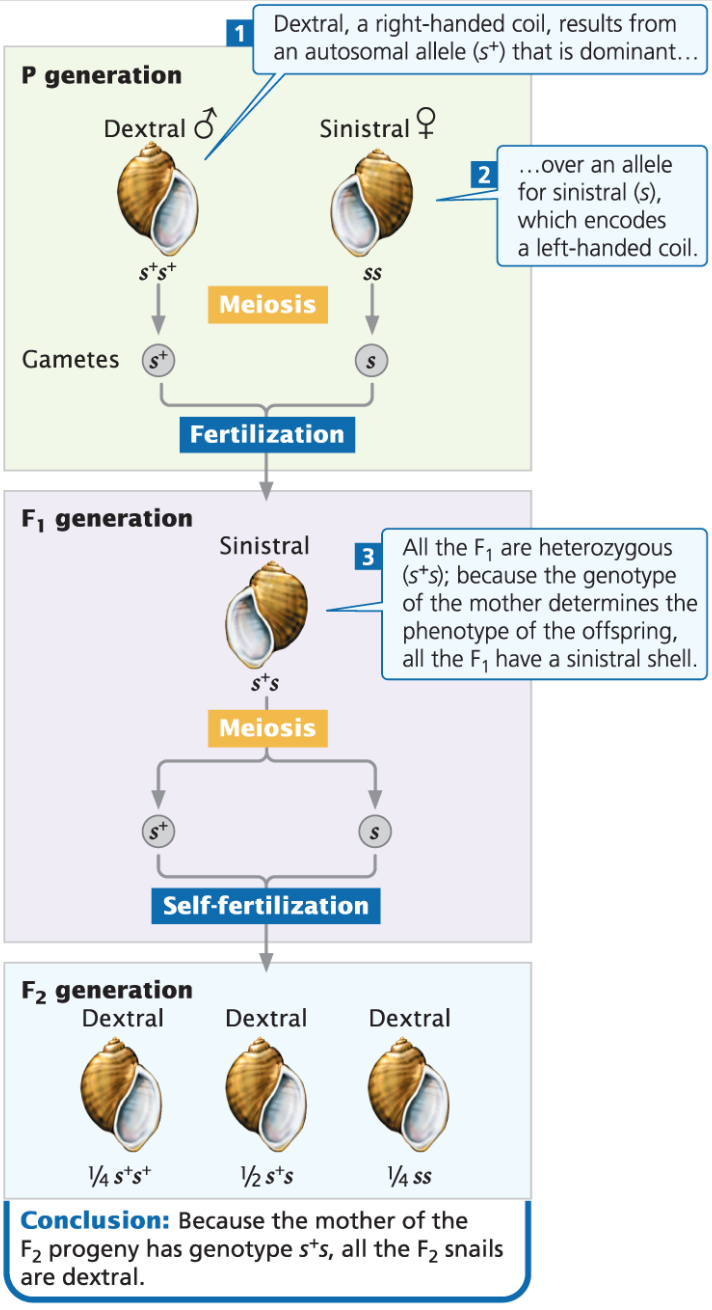
eyes, which results from the death of cells in the optic nerve. This loss of vision typically occurs in early adulthood (usually between the ages of 20 and 24), but it can occur any time after adolescence. There is much clinical variation in the severity of the disease, even within the same family. Leber hereditary optic neuropathy exhibits cytoplasmic inheritance: the trait is passed from a mother to all her children, sons and daughters alike.

Genetic Maternal Effect

A genetic phenomenon that is sometimes confused with cytoplasmic inheritance is **genetic maternal effect**, in which the phenotype of the offspring is determined by the genotype of the mother. In cytoplasmic inheritance, the genes for a characteristic are inherited from only one parent, usually the mother. In genetic maternal effect, the genes are inherited from both parents, but the offspring's phenotype is determined not by its own genotype but by the genotype of its mother.

Genetic maternal effect frequently arises when substances present in the cytoplasm of an egg (encoded by the mother's nuclear genes) are pivotal in early development. An excellent example is the direction of shell coiling in the snail *Lymnaea peregra* (**Figure 5.17**), described in the introduction to this chapter. The direction of coiling is determined by a pair of alleles; the allele for dextral (right-handed) coiling (s^+) is dominant to the allele for sinistral (left-handed) coiling

(*s*). However, the direction of coiling is determined not by a snail's own genotype but by the genotype of its mother. The direction of coiling is affected by the way in which the egg cytoplasm divides soon after fertilization, which in turn is determined by a substance produced by the mother and passed to the offspring in the cytoplasm of the egg.



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5.17 In genetic maternal effect, the genotype of the maternal parent determines the phenotype of the offspring. Chirality (the direction of shell coiling) in the snail *Lymnaea peregra* is a characteristic that exhibits genetic maternal effect.

Description
In generation P on the top, a dextral male snail with genotype small S superscript positive small S superscript positive is crossed with a sinistral female with genotype small S small S. A caption reads, “Dextral, a right-handed coil, results from an autosomal allele (small S superscript positive) that is dominant over an allele for sinistral (small S), which encodes a left-handed coil.” Meiosis results in the formation of gametes as follows: small S superscript positive from the male and small S from the female. Fertilization occurs and leads to the big F subscript 1 generation below which has sinistral offspring with genotype small S superscript positive small S. A caption referring to the progeny reads, “All the big F subscript 1 are heterozygous (small S superscript positive small S); because the genotype of the mother determines the phenotype of the offspring, all the big F subscript 1 have a sinistral shell.” This progeny produced in F subscript 1 generation undergoes meiosis and forms two gametes with genotype small S superscript positive and small S respectively which are self-fertilized. This self-fertilization results in the production of progeny subscript 2 generation below. The progeny are as follows: one-fourth dextral with genotype small S superscript positive small S superscript positive; one-half dextral with genotype S superscript positive small S; and one-fourth dextral with genotype small S small S. A caption reads, Conclusion: Because the mother of the big F subscript 2 progeny has genotype small s superscript positive small s, all the F subscript 2 snails are dextral.

If a male homozygous for dextral alleles ($s^+ s^+$) is crossed with a female homozygous for sinistral alleles (ss), all the F_1 are heterozygous ($s^+ s$), but they all have sinistral shells because the genotype of the mother (ss) encodes sinistral coiling (see Figure 5.17). If these F_1 snails self-fertilize, the genotypic ratio of the F_2 is 1 $s^+ s^+$: 2 $s^+ s$: 1 ss. Notice, however, that the phenotype of all the F_2 snails is dextral, regardless of their genotypes. The F_2 offspring are dextral because the genotype of their mother ($s^+ s$), which encodes a dextral shell, determines their phenotype. With genetic maternal effect, the phenotype of the progeny is not necessarily the same as

the phenotype of the mother because the progeny's phenotype is determined by the mother's *genotype*, not her phenotype. Neither the male parent's nor the offspring's own genotype has any role in the offspring's phenotype. However, a male does influence the phenotype of the F_2 generation: by contributing to the genotypes of his daughters, he affects the phenotypes of their offspring. Genes that exhibit genetic maternal effect are therefore transmitted through males to future generations. In contrast, genes that exhibit cytoplasmic inheritance are transmitted through only one of the sexes (usually the female). › **TRY** PROBLEM 39

CONCEPTS

Characteristics exhibiting cytoplasmic inheritance are encoded by genes in the cytoplasm and are usually inherited from one parent, most commonly the mother. In genetic maternal effect, the genotype of the mother determines the phenotype of the offspring.

✔ CONCEPT CHECK 10

How might you determine whether a particular trait is due to cytoplasmic inheritance or to genetic maternal effect?

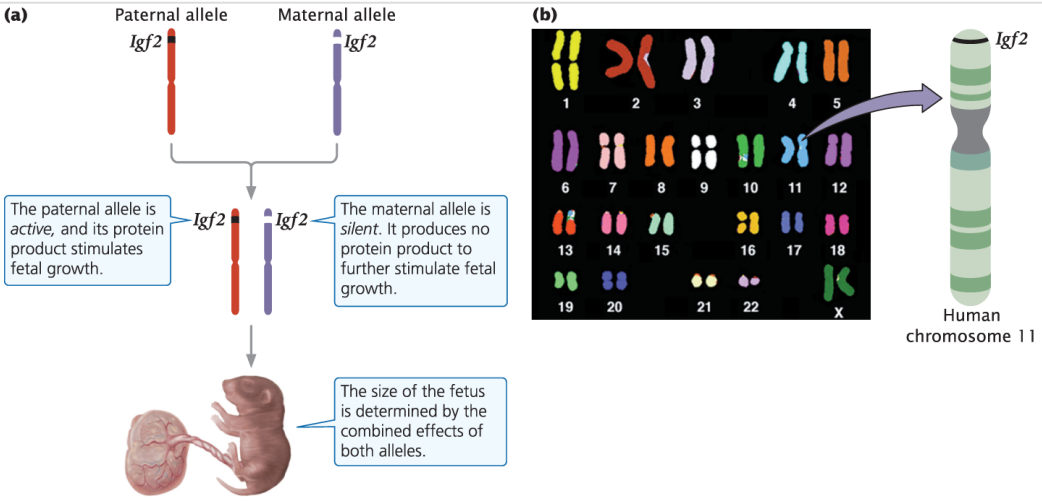
Genomic Imprinting

A basic tenet of Mendelian genetics is that the parental origin of a gene does not affect its expression and, therefore, reciprocal crosses

give identical results. We have seen, however, that there are some genetic characteristics—those encoded by X-linked genes and cytoplasmic genes—for which reciprocal crosses do not give the same results. In these cases, males and females do not contribute the same genetic material to the offspring. Males and females do contribute the same number of autosomal genes to their offspring, and paternal and maternal autosomal genes have long been assumed to have equal effects. However, the expression of some autosomal genes is significantly affected by their parental origin. This differential expression of genetic material depending on whether it is inherited from the male or the female parent is called **genomic imprinting**. Although the number of imprinted genes is unknown, recent research suggests that more genes may be imprinted than previously suspected (see Chapter 21).

A gene that exhibits genomic imprinting in both mice and humans is *Igf2*, which encodes a protein called insulin-like growth factor 2 (Igf2). Offspring inherit one *Igf2* allele from their mother and one from their father. The paternal copy of *Igf2* is actively expressed in the fetus and placenta, but the maternal copy is completely silent (**Figure 5.18**). Both male and female offspring possess *Igf2* genes; the key to whether the gene is expressed is the sex of the parent transmitting the gene. In the present example, the gene is expressed only when it is transmitted by a male parent. In some way that is not completely understood, the paternal *Igf2* allele (but not the maternal allele) promotes placental and fetal growth; when the paternal copy

of *Igf2* is deleted in mice, a small placenta and low-birth-weight offspring result. In other genomically imprinted traits, only the copy of the gene transmitted by the female parent is expressed.



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Courtesy of Dr. Thomas Ried and Dr. Evelin Schrock.

5.18 Genomic imprinting of the *Igf2* gene in mice and humans affects fetal growth. (a) The paternal *Igf2* allele is active in the fetus and placenta, whereas the maternal allele is silent. (b) The human *Igf2* locus is on the short arm of chromosome 11; the locus in mice is on chromosome 7.

Description

Part “a” on the left shows the paternal allele with big I small G small F 2 locus on a red chromosome with a black bar, indicating it is active. The maternal chromosome is blue and the big I small G small F 2 locus is colored white. A caption referring to the paternal allele reads, “The paternal allele is active and its protein product stimulates fetal growth.” Another caption referring to the maternal allele reads, “The maternal allele is silent. It produces no protein product to further stimulate fetal growth.” At the bottom of the figure is a picture of a mouse fetus along with a caption that reads, “The size of the fetus is determined by the combined effects of both alleles.”

Part “b” shows a picture of the human genome hybridized with fluorescent probes and an arrow from chromosome 11 to a drawing of chromosome 11 with the I small G small F 2 gene labeled. This gene is represented as a black band near the tip of its short arm.

Genomic imprinting has been implicated in several human disorders, including Prader–Willi and Angelman syndromes. Children with Prader–Willi syndrome have small hands and feet, short stature, poor sexual development, and intellectual disability. These children are small at birth and nurse poorly, but as toddlers they develop voracious appetites and frequently become obese. Many people with Prader–Willi syndrome are missing a small region on the long arm of chromosome 15. The deletion of this region is always inherited from the *father*. Thus, children with Prader–Willi syndrome lack a paternal copy of genes on the long arm of chromosome 15.

The deletion of this same region of chromosome 15 can also be inherited from the *mother*, but this inheritance results in a completely different set of symptoms, producing Angelman syndrome. Children with Angelman syndrome exhibit frequent laughter, uncontrolled muscle movement, a large mouth, and unusual seizures. They are missing a maternal copy of genes on the long arm of chromosome 15. For normal development to take place, copies of this region of chromosome 15 from both male and female parents are apparently required.

Many imprinted genes in mammals are associated with fetal growth. Imprinting has also been reported in plants, with differential expression of paternal and maternal genes in the endosperm, which, like the placenta in mammals, provides nutrients for the growth of

the embryo. The mechanism of imprinting is still under investigation, but the methylation of DNA—the addition of methyl (CH_2) groups to DNA nucleotides (see Chapters 10 and 17)—is essential to the process. In mammals, methylation is erased in the germ cells each generation and then reestablished in the course of gamete formation, with sperm and eggs undergoing different levels of methylation, which then cause the differential expression of male and female alleles in the offspring. Some of the different ways in which sex interacts with heredity are summarized in **Table 5.5**.

TABLE 5.5 Influences of sex on heredity

Genetic Phenomenon	Phenotype Determined by
Sex-linked characteristic	Genes located on the sex chromosomes
Sex-influenced characteristic	Autosomal genes that are more readily expressed in one sex
Sex-limited characteristic	Autosomal genes whose expression is limited to one sex
Genetic maternal effect	Nuclear genotype of the maternal parent
Cytoplasmic inheritance	Cytoplasmic genes, which are usually inherited from only one parent

Genomic imprinting	Genes whose expression is affected by the sex of the transmitting parent

EPIGENETICS

Genomic imprinting is just one form of a phenomenon known as **epigenetics**. Most traits are encoded by genetic information that resides in the sequence of the nucleotide bases of DNA—the genetic code, which will be discussed in Chapter 15. However, some traits are determined by modifications to DNA or chromatin—such as DNA methylation—that affect the way in which DNA sequences are expressed. These changes are often stable and heritable in the sense that they are passed from one cell to another. For example, in genomic imprinting, whether a gene passes through the egg or the sperm determines how much methylation of the DNA takes place. The pattern of methylation on a gene is copied when the DNA is replicated and therefore remains on the gene as it is passed from cell to cell through mitosis. However, the methylation may be modified or removed when the DNA passes through a gamete, so a gene that is methylated in sperm may be unmethylated when it is eventually passed down to a daughter's egg. Ultimately, the amount of methylation determines whether the gene is expressed in the offspring.

These types of reversible changes to DNA that influence the expression of traits are termed epigenetic marks. The inactivation of

one of the X chromosomes in female mammals (discussed in Chapter 4) is another type of epigenetic change. We will consider epigenetic changes in more detail in Chapter 21.

CONCEPTS

In genomic imprinting, the expression of a gene is influenced by the sex of the parent transmitting the gene to the offspring. Epigenetic marks are reversible changes in DNA that do not alter the base sequence but may affect how a gene is expressed.

✔ CONCEPT CHECK 11

What type of epigenetic mark is responsible for genomic imprinting?

5.4 Anticipation Is the Stronger or Earlier Expression of Traits in Succeeding Generations

Another genetic phenomenon that is not explained by Mendel's principles is **anticipation**, in which a genetic trait becomes more strongly expressed, or is expressed at an earlier age, as it is passed from generation to generation. In the early 1900s, several physicians observed that many patients with moderate to severe myotonic dystrophy—an autosomal dominant muscle disorder—had ancestors who were only mildly affected by the disease. These observations led to the concept of anticipation. The concept quickly fell out of favor with geneticists, however, because there was no obvious mechanism to explain it; traditional genetics held that genes are passed unaltered from parents to offspring. Geneticists tended to attribute anticipation to observational bias.

Research has now reestablished anticipation as a legitimate genetic phenomenon. The mutation that causes myotonic dystrophy consists of an unstable region of DNA that can increase in size as the gene is passed from generation to generation. The age of onset and the severity of the disease are correlated with the size of the unstable region; an increase in the size of the region over generations produces anticipation. The phenomenon has now been

implicated in a number of genetic diseases. We will examine these interesting types of mutations in more detail in Chapter 18.

CONCEPTS

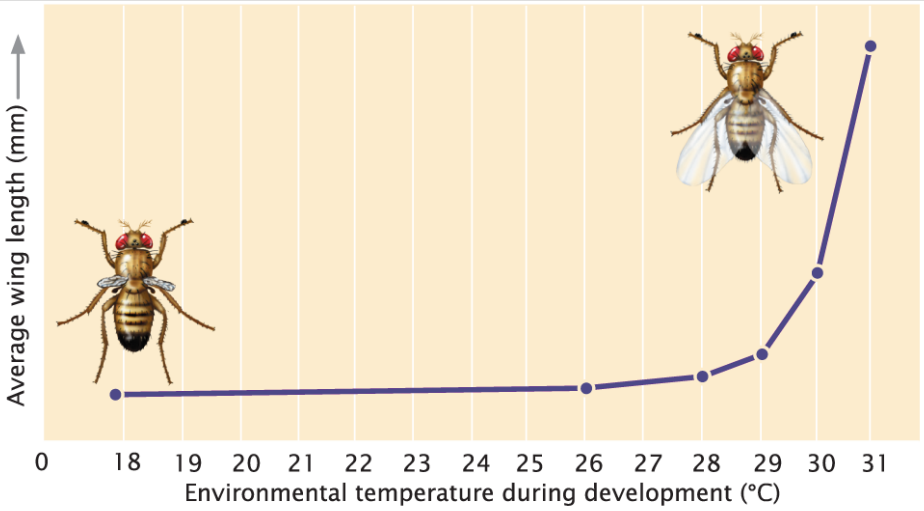
Anticipation is the stronger or earlier expression of a genetic trait in succeeding generations. It is caused by an unstable region of DNA that increases in size from generation to generation.

5.5 Phenotypes May Be Influenced by Environmental Factors and Multiple Genes

Often, phenotypes are not the result of a variation at a single gene but instead are influenced by environmental factors and multiple loci.

Environmental Effects on the Phenotype

In Chapter 3, we learned that each phenotype is the result of a genotype that develops within a particular environment; each genotype may produce several different phenotypes, depending on the environmental conditions in which development takes place. For example, a fruit fly that is homozygous for the *vestigial* mutation (*vg vg*) develops reduced wings when raised at a temperature below 29°C, but the same genotype develops much longer wings when raised at 31°C (**Figure 5.19**).



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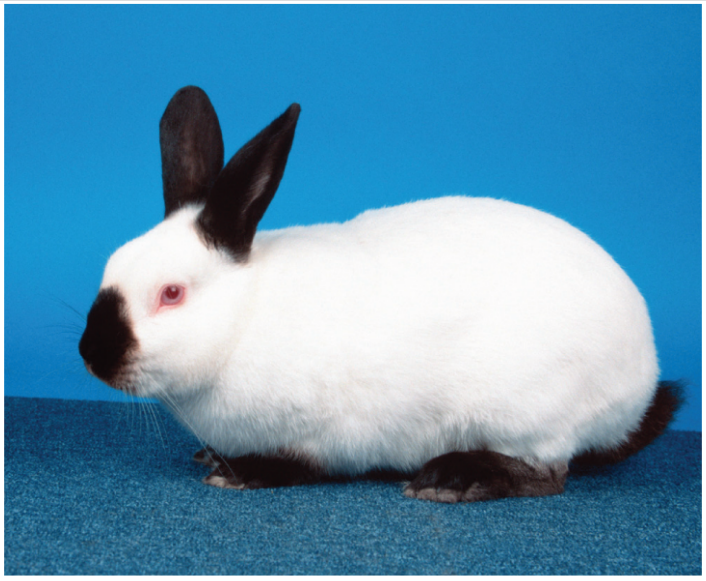
5.19 The expression of the *vestigial* mutation in *Drosophila* is temperature dependent. When reared at temperatures below 29°C, flies homozygous for *vestigial* have greatly reduced wings; at temperatures above 31°C, the flies develop normal wings. [Data from M. H. Harnly, *Journal of Experimental Zoology* 56:363–379, 1936.]

Description

The horizontal axis represents environmental temperature during development in degree Celsius ranging from 18 to 31 with an interval of 1. The vertical axis represents average wing length in m m and begins from 0. The curve starts above 18 degree Celsius, remains constant till it approaches 26 degree Celsius, rises gradually as it reaches 29 degree Celsius, and eventually rises exponentially as it approaches 31 degree Celsius. The fly has no wings when the curve begins and has wings as the curve rises towards the end of the graph. All values are approximate.

For most of the characteristics we have discussed so far, the effect of the environment on the phenotype has been slight. Mendel's peas with genotype *yy*, for example, developed green seeds regardless of the environment in which they were raised. Similarly, people with genotype $I^A I^A$ have the A antigen on their red blood cells regardless

of their diet, socioeconomic status, or family environment. For other phenotypes, however, environmental effects play a more important role. The phenotypic expression of some genotypes depends critically on the presence of a specific environment. For example, the *himalayan* allele in rabbits produces dark fur at the extremities of the body—on the nose, ears, and feet (**Figure 5.20**). The dark pigment develops, however, only when a rabbit is reared at a temperature of 25°C or lower; if a Himalayan rabbit is reared at 30°C, no dark patches develop. The expression of the *himalayan* allele is thus temperature dependent; an enzyme necessary for the production of dark pigment is inactivated at higher temperatures. The pigment is restricted to the nose, ears, and feet of a Himalayan rabbit because the animal's core body temperature is normally above 25°C and the enzyme is functional only in the cells of the relatively cool extremities. The *himalayan* allele is an example of a **temperature-sensitive allele**, an allele whose product is functional only at certain temperatures. Similarly, vestigial wings in *Drosophila melanogaster* are caused by a temperature-dependent mutation (see Figure 5.19).



Petra Wegner/Alamy.

5.20 The expression of the *himalayan* allele is temperature dependent. This rabbit was reared below 25°C. Its pigment is restricted to the extremities where the body temperature falls below 25°C and the enzyme that produces pigment is functional.

Environmental factors also play an important role in the expression of a number of human genetic diseases. Phenylketonuria (PKU) is due to an autosomal recessive allele that causes intellectual disability. The disorder arises from a defect in an enzyme that normally metabolizes the amino acid phenylalanine. When this enzyme is defective, phenylalanine is not metabolized, and its buildup causes neurological damage in children. A simple environmental change, putting an affected child on a low-phenylalanine diet, prevents the development of intellectual disability.

THINK-PAIR-SHARE Question 6

These examples illustrate the point that genes and their products do not act in isolation; rather, they frequently interact with environmental factors. Occasionally, environmental factors alone can produce a phenotype that is the same as the phenotype produced by a genotype; such a phenotype is called a **phenocopy**. In fruit flies, for example, the autosomal recessive mutation *eyeless* produces greatly reduced eyes. The eyeless phenotype can also be produced by exposing the larvae of normal flies to sodium metaborate.

CONCEPTS

The expression of many genes is modified by the environment. A phenocopy is a phenotype produced by environmental effects that mimics the phenotype produced by a genotype.

✔ CONCEPT CHECK 12

How can you determine whether a phenotype such as reduced eyes in fruit flies is due to a recessive mutation or is a phenocopy?

The Inheritance of Continuous Characteristics

So far, we've dealt primarily with characteristics that have only a few distinct phenotypes. In Mendel's peas, for example, the seeds were either smooth or wrinkled, yellow or green; the coats of dogs were

black, brown, or yellow; blood types were A, B, AB, or O. Such characteristics, which have a few easily distinguished phenotypes, are called **discontinuous characteristics**.

Not all characteristics exhibit discontinuous phenotypes, however. Human height is an example; people do not come in just a few distinct heights but rather display a wide range of heights. Indeed, there are so many possible phenotypes of human height that we must use a measurement to describe a person's height. Such characteristics are said to exhibit a continuous distribution of phenotypes, and they are termed **continuous characteristics**. Because they have many possible phenotypes and must be described in quantitative terms, continuous characteristics are also called **quantitative characteristics**.

Continuous characteristics frequently arise because genes at many loci interact to produce the phenotypes. When a single locus with two alleles encodes a characteristic, there are three genotypes possible: *AA*, *Aa*, and *aa.* With two loci, each with two alleles, there are $3^2 = 9$ genotypes possible. The number of genotypes encoding a characteristic is 3^n, where n equals the number of loci, each with two alleles, that influence the characteristic. For example, when a characteristic is determined by eight loci, each with two alleles, there are $3^8 = 6561$ different genotypes possible for this characteristic. If each genotype produces a different phenotype, many phenotypes will be possible. The slight differences between the phenotypes will be indistinguishable, and the characteristic will

appear to be continuous. Characteristics encoded by genes at many loci are called **polygenic characteristics**.

The converse of polygeny is **pleiotropy**, in which one gene affects multiple characteristics. Many genes exhibit pleiotropy. Phenylketonuria, mentioned earlier, results from a recessive allele; people homozygous for this allele, if untreated, exhibit intellectual disability, blue eyes, and light skin color. The lethal allele that causes yellow coat color in mice is also pleiotropic. In addition to its lethality and its effect on coat color, the gene causes a diabetes-like condition, obesity, and an increased propensity to develop tumors.

The phenotypes of many continuous characteristics are also influenced by environmental factors. In these cases, each genotype is capable of producing a range of phenotypes, and the particular phenotype that results depends on both the genotype and the environmental conditions in which the genotype develops. For example, there may be only three genotypes at a single locus, but because each genotype produces a range of phenotypes associated with different environments, the phenotype of the characteristic will exhibit a continuous distribution. Many continuous characteristics are both polygenic and influenced by environmental factors; such characteristics are called **multifactorial characteristics** because many factors help determine the phenotype. Human height is an example of a multifactorial characteristic; variation in height is determined by a number of genes as well as environmental factors such as nutrition.

The inheritance of continuous characteristics may appear to be complex, but the alleles at each locus follow Mendel's principles and are inherited in the same way as alleles encoding simple, discontinuous characteristics. However, because many genes are involved, because environmental factors influence the phenotype, and because the phenotypes do not sort out into a few distinct types, we cannot observe the distinct ratios that have allowed us to interpret the genetic basis of discontinuous characteristics. To analyze continuous characteristics, we must employ special statistical tools, which will be discussed in Chapter 24. › **TRY PROBLEM 45**

CONCEPTS

Discontinuous characteristics exhibit a few distinct phenotypes; continuous characteristics exhibit a range of phenotypes. A continuous characteristic is frequently produced when genes at many loci and environmental factors combine to determine a phenotype.

✔ CONCEPT CHECK 13

What is the difference between polygeny and pleiotropy?

Chapter 5 Review

CHAPTER SUMMARY

Section 5.1 Learning Objective: Be able to work and interpret genetic crosses involving factors that affect inheritance at a single locus, including different types of dominance, incomplete penetrance, lethal alleles, and multiple alleles.

- Dominance is an interaction between genes at the same locus (allelic genes) and can be understood in regard to how the phenotype of the heterozygote relates to the phenotypes of the homozygotes.
- Dominance is complete when a heterozygote has the same phenotype as one homozygote. It is incomplete when the heterozygote has a phenotype intermediate between those of two parental homozygotes. In codominance, the heterozygote exhibits traits of both parental homozygotes.
- The type of dominance does not affect the inheritance of an allele; it does affect the phenotypic expression of the allele. The classification of dominance depends on the level at which the phenotype is examined.
- Penetrance is the percentage of individuals having a particular genotype that exhibit the expected phenotype. Expressivity is the degree to which a character is expressed.
- Lethal alleles cause the death of an individual possessing them at an early stage of development and may alter the phenotypic

ratios resulting from a cross.
- The existence of multiple alleles refers to the presence of more than two alleles at a locus within a group of individuals. The presence of multiple alleles increases the number of genotypes and phenotypes that are possible.

Section 5.2 Learning Objective: Be able to work and interpret genetic crosses involving gene interaction.

- Gene interaction refers to interaction between genes at different loci to produce a single phenotype. An epistatic gene at one locus suppresses, or masks, the expression of hypostatic genes at other loci. Gene interaction frequently produces phenotypic ratios that are modifications of dihybrid ratios.

Section 5.3 Learning Objective: Be able to work and interpret genetic crosses in which sex influences the inheritance and expression of genes.

- Sex-influenced characteristics are encoded by autosomal genes that are expressed more readily in one sex. Sex-limited characteristics are encoded by autosomal genes that are expressed in only one sex.
- In cytoplasmic inheritance, the genes that encode a characteristic are found in the organelles and are usually inherited from a single (typically maternal) parent. In genetic maternal effect, an offspring inherits genes from both parents, but the nuclear genes of the mother determine the offspring's phenotype.

- Genomic imprinting refers to differential expression of autosomal genes depending on the sex of the parent transmitting the genes. Epigenetic effects such as genomic imprinting are caused by alterations to DNA—such as DNA methylation—that do not affect the DNA base sequence.

Section 5.4 Learning Objective: Be able to explain anticipation.

- Anticipation refers to the stronger expression of a genetic trait, or its expression at an earlier age, in succeeding generations.

Section 5.5 Learning Objective: Be able to describe how environmental effects and multiple genes influence the expression of some traits.

- Phenotypes are often modified by environmental effects. A phenocopy is a phenotype produced by an environmental effect that mimics a phenotype produced by a genotype.
- Continuous characteristics are those that exhibit a wide range of phenotypes; they are frequently produced by the combined effects of many genes and environmental effects.

WORKED PROBLEMS

Problem 1

A geneticist crosses two yellow mice with straight hair and obtains the following progeny:

$^1/_2$	yellow, straight
$^1/_6$	yellow, fuzzy
$^1/_4$	gray, straight
$^1/_{12}$	gray, fuzzy

a. Provide a genetic explanation for the results, and assign genotypes to the parents and progeny of this cross.
b. What additional crosses might be carried out to determine if your explanation is correct?

» Solution Strategy

What information is required in your answer to the problem?

a. A genetic explanation for the inheritance of color and hair type in the mice. Genotypes of the parents.
b. Examples of other crosses that might be carried out to determine if the explanation given in your answer to part *a* is correct.

What information is provided to solve the problem?

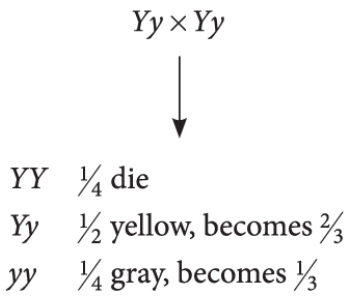
- Phenotypes of the parents.
- Phenotypes and proportions of different types of progeny.

For help with this problem, review:

Lethal Alleles in Section 5.1 and ratios for simple genetic crosses (Table 3.5).

» Solution Steps

a. This cross concerns two separate characteristics—color and hair type. First, let's look at the inheritance of color. Two yellow mice are crossed, producing $^1/_2 + {}^1/_6 = {}^3/_6 + {}^1/_6 = {}^4/_6 = {}^2/_3$ yellow mice and $^1/_4 + {}^1/_{12} = {}^3/_{12} + {}^1/_{12} = {}^4/_{12} = {}^1/_3$ gray mice. We learned in this chapter that a 2:1 ratio is often produced when a recessive lethal allele is present:

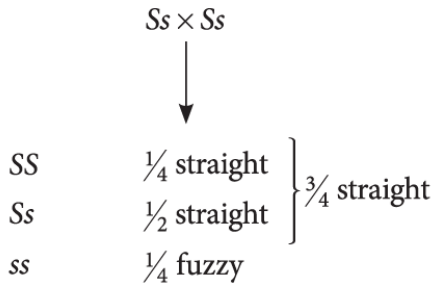


Description
The illustration shows a mice with genotype big Y small Y is intercrossed with another mice of same genotype. A downward arrow points to the progenies produced as follows: big Y big Y, one-fourth die; big Y small Y: one-half yellow, becomes two-third; small Y small Y: one-fourth gray, becomes one-third.

Hint: Examine the progeny ratios for each trait separately.

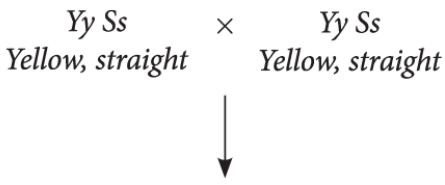
Recall: A 2 : 1 ratio is usually produced by a lethal allele.

Now, let's examine the inheritance of the hair type. Two mice with straight hair are crossed, producing $^1/_2 + {}^1/_4 = {}^2/_4 + {}^1/_4 = {}^3/_4$ mice with straight hair and $^1/_6 + {}^1/_{12} = {}^2/_{12} + {}^1/_{12} = {}^3/_{12} = {}^1/_4$ mice with fuzzy hair. We learned in Chapter 3 that a 3:1 ratio is usually produced by a cross between two individuals heterozygous for a simple dominant allele:



Description
The illustration shows a parent with genotype big S small S is intercrossed with another parent of same genotype. A downward arrow points to the progenies produced: big S big S one-fourth straight; big S small S one-half straight; small S small S: one-fourth fuzzy. Three-fourth have straight hair.

We can now combine both loci and assign genotypes to all the individual mice in the cross:



Phenotype	Genotype	Probability at each locus	Combined probability
yellow, straight	*Yy S_*	$\frac{2}{3} \times \frac{3}{4}$	$= \frac{6}{12} = \frac{1}{2}$
yellow, fuzzy	*Yy ss*	$\frac{2}{3} \times \frac{1}{4}$	$= \frac{2}{12} = \frac{1}{6}$
gray, straight	*yy S_*	$\frac{1}{3} \times \frac{3}{4}$	$= \frac{3}{12} = \frac{1}{4}$
gray, fuzzy	*yy ss*	$\frac{1}{3} \times \frac{1}{4}$	$= \frac{1}{12}$

Description
The illustration shows two mice with yellow, straight hair crossing. The progenies produced are present below. The phenotype, genotype, probability at each locus, and combined probability. This data are written in a table format as follows:

Phenotype: yellow straight; genotype: big Y small Y big S underscore; probability at each locus: two-third multiplied by three-fourth; combined probability equals six over twelve equals one-half.

Phenotype: yellow fuzzy; genotype: big Y small Y small S small S; probability at each locus: two-third multiplied by one-fourth; combined probability equals two over twelve equals one-sixth.

Phenotype: gray straight; genotype: small Y small Y big S underscore; probability at each locus: one-third multiplied by three-fourth; combined probability equals three over twelve equals one-fourth.

Phenotype: gray fuzzy; genotype: small Y small Y small S small S; probability at each locus: one-third multiplied by one-fourth; combined probability equals one over twelve.

b. We could carry out a number of different crosses to test our hypothesis that yellow is a recessive lethal and straight is dominant to fuzzy. For example, a cross between any two yellow mice should always produce $^2/_3$ yellow and $^1/_3$ gray offspring,

and a cross between two gray mice should produce only gray offspring. A cross between two fuzzy mice should produce only fuzzy offspring.

Problem 2

In some sheep, horns are produced by an autosomal allele that is dominant in males and recessive in females. A horned female is crossed with a hornless male. One of the resulting F_1 females is crossed with a hornless male. What proportion of the male and female progeny from this cross will have horns?

» Solution Strategy

What information is required in your answer to the problem?

Proportions of male and female progeny that have horns.

What information is provided to solve the problem?

- The presence of horns is due to an autosomal gene that is dominant in males and recessive in females.
- A horned female is crossed with a hornless male. A resulting F_1 female is crossed with a hornless male to produce progeny.

For help with this problem, review:

Sex-Influenced and Sex-Limited Characteristics in Section 5.3.

» Solution Steps

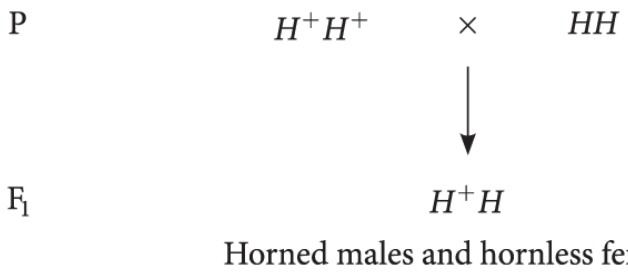
The presence of horns in these sheep is an example of a sex-influenced characteristic. Because the phenotypes associated with the genotypes differ between the two sexes, let's begin this problem by writing out the genotypes and phenotypes for each sex. We will let H represent the allele that encodes horns and H^+ represent the allele that encodes hornless. In males, the allele for horns is dominant to the hornless allele, which means that males homozygous (HH) and heterozygous ($H^+ H$) for this gene are horned. Only males homozygous for the recessive hornless allele ($H^+ H^+$) are hornless. In females, the allele for horns is recessive, which means that only females homozygous for this allele (HH) are horned; females heterozygous ($H^+ H$) and homozygous ($H^+ H^+$) for the hornless allele are hornless. The following table summarizes the genotypes and their associated phenotypes:

Genotype	Male phenotype	Female phenotype
HH	horned	horned
HH^+	horned	hornless
$H^+ H^+$	hornless	hornless

Hint: Write out the genotypes and the associated phenotype for each sex.

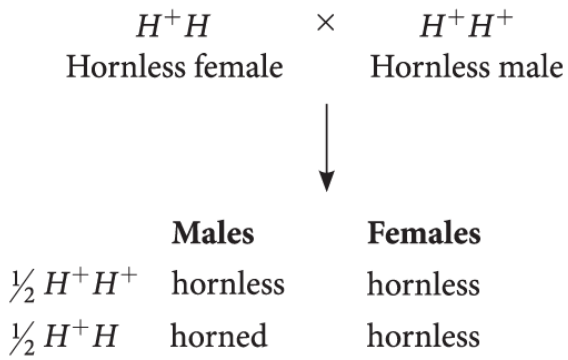
Recall: When a trait is dominant, both the homozygote and the heterozygote express the trait in their phenotypes.

In the problem, a horned female is crossed with a hornless male. From the preceding table, we see that a horned female must be homozygous for the allele for horns (*HH*), and that a hornless male must be homozygous for the hornless allele ($H^+ H^+$), so all the F_1 will be heterozygous; the F_1 males will be horned and the F_1 females will be hornless, as shown in the following diagram:



Description
The illustration shows cross between parents (P) with genotypes, big H superscript positive big H superscript positive and big H big H. The cross produces the following progeny in in F subscript 1 generation below: horned males and hornless females with genotype, big H superscript positive big H.

A heterozygous hornless F_1 female ($H^+ H$) is then crossed with a hornless male ($H^+ H^+$):



Description
The illustration shows a hornless female with genotype big H superscript positive big H is crossed with a hornless female with genotype big H superscript positive big H superscript positive. The cross produces the following progenies below: one-half hornless males with genotype big H superscript positive big H superscript positive; one-half hornless females with genotype big H superscript positive big H superscript positive; one-half hornless females with genotype big H superscript positive big H; and one-half horned males with genotype big H superscript positive big H.

Therefore, $^1/_2$ of the male progeny will be horned, but none of the female progeny will be horned.

IMPORTANT TERMS

complete dominance
incomplete dominance
codominance
incomplete penetrance
penetrance
expressivity
lethal allele

multiple alleles
compound heterozygote
gene interaction
epistasis
epistatic gene
hypostatic gene
complementation test
complementation
sex-influenced characteristic
sex-limited characteristic
cytoplasmic inheritance
genetic maternal effect
genomic imprinting
epigenetics
anticipation
temperature-sensitive allele
phenocopy
discontinuous characteristic
continuous characteristic
quantitative characteristic
polygenic characteristic
pleiotropy
multifactorial characteristic

ANSWERS TO CONCEPT CHECKS

1. b

2. With complete dominance, the heterozygote expresses the same phenotype as one of the homozygotes. With incomplete dominance, the heterozygote has a phenotype that is intermediate between those of the two homozygotes. With codominance, the heterozygote has a phenotype that simultaneously expresses the phenotypes of both homozygotes.
3. d
4. b
5. c
6. Gene interaction is interaction between genes at different loci. Dominance is interaction between alleles at a single locus.
7. d
8. Cross a bulldog homozygous for *brindle* with a Chihuahua homozygous for *brindle*. If the two *brindle* genes are allelic, all the offspring will be brindle: $bb \times bb \rightarrow$ all bb (brindle). If, on the other hand, brindle in the two breeds is due to recessive genes at different loci, then none of the offspring will be brindle: $a^+a^+ bb \times aa\ \mathrm{b}^+\mathrm{b}^+ \rightarrow a^+a\ b^+b$.
9. Both sex-influenced and sex-limited characteristics are encoded by autosomal genes whose expression is affected by the sex of the individual organism possessing the genes. Sex-linked characteristics are encoded by genes on the sex chromosomes.
10. Cytoplasmically inherited traits are encoded by genes in the cytoplasm, which is usually inherited only from the female parent. Therefore, a trait due to cytoplasmic inheritance will always be passed through females. Traits due to genetic maternal effect are encoded by autosomal genes and can therefore be passed through males, although any individual

organism's trait is determined by the genotype of the maternal parent.

11. Methylation of DNA.
12. Cross two eyeless flies and cross an eyeless fly with a wild-type fly. Raise the offspring of both crosses in the same environment. If the trait is due to a recessive mutation, all the offspring of the two eyeless flies should be eyeless, whereas at least some of the offspring of the eyeless and wild-type flies should be wild type. If the trait is a phenocopy, there should be no differences in the progeny of the two crosses.
13. Polygeny refers to the influence of multiple genes on the expression of a single characteristic. Pleiotropy refers to the effect of a single gene on the expression of multiple characteristics.

Chapter 5 Assessment

COMPREHENSION QUESTIONS

Section 5.1

1. What are some important characteristics of dominance?
2. What is incomplete penetrance, and what causes it?

Section 5.2

3. What is gene interaction? What is the difference between an epistatic gene and a hypostatic gene?
4. What is a recessive epistatic gene?
5. What is a complementation, test and what is it used for?

Section 5.3

6. What characteristics are exhibited by a cytoplasmically inherited trait?
7. What is genomic imprinting?
8. What is the difference between genetic maternal effect and genomic imprinting?

9. What is the difference between a sex-influenced gene and a gene that exhibits genomic imprinting?

Section 5.4

10. What characteristics do you expect to see in a trait that exhibits anticipation?

Section 5.5

11. What are continuous characteristics, and how do they arise?

APPLICATION QUESTIONS AND PROBLEMS

Sections 5.1 through 5.4

12. Match each of the following terms with its correct definition (parts *a* through *i*).

    _____ Phenocopy
    _____ Pleiotropy
    _____ Polygenic trait
    _____ Penetrance
    _____ Sex-limited trait
    _____ Genetic maternal effect
    _____ Genomic imprinting
    _____ Sex-influenced trait
    _____ Anticipation

a. The percentage of individuals with a particular genotype that express the expected phenotype.
b. A trait determined by an autosomal gene that is more easily expressed in one sex.
c. A trait determined by an autosomal gene that is expressed in only one sex.
d. A trait that is determined by an environmental effect and that has the same phenotype as a genetically determined trait.
e. A trait determined by genes at many loci.
f. The expression of a trait is affected by the sex of the parent that transmits the gene to the offspring.
g. The trait appears earlier or is more severe in succeeding generations.
h. A gene affects more than one phenotype.
i. The genotype of the maternal parent influences the phenotype of the offspring.

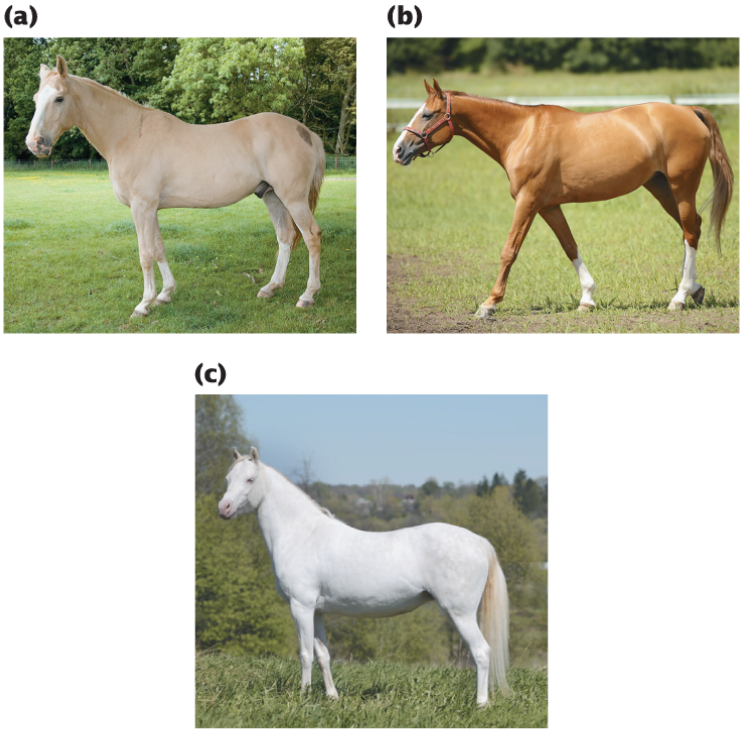
Section 5.1

13. *Palomino horses have a golden yellow coat, chestnut horses have a brown coat, and cremello horses have a coat that is almost white. A series of crosses between the three different types of horses produced the following offspring:

Cross	Offspring
palomino × palomino	13 palomino, 6 chestnut, 5 cremello
chestnut × chestnut	16 chestnut

cremello × cremello	13 cremello
palomino × chestnut	8 palomino, 9 chestnut
palomino × cremello	11 palomino, 11 cremello
chestnut × cremello	23 palomino

a. Explain the inheritance of the palomino, chestnut, and cremello phenotypes in horses.
b. Assign symbols for the alleles that determine these phenotypes, and list the genotypes of all parents and offspring given in the preceding table.



Part a: Keith J. Smith/Alamy. Part b: jirijura/Getty Images.
Part c: Olga_i/Shutterstock.

Coat color: (a) palomino; (b) chestnut; (c) cremello.

14. The L^M and L^N alleles at the MN blood-group locus exhibit codominance. Give the expected genotypes and phenotypes and their ratios in progeny resulting from the following crosses.

    a. $L^ML^M \times L^ML^N$

    b. $L^NL^N \times L^NL^N$

    c. $L^ML^N \times L^ML^N$

    d. $L^ML^N \times L^NL^N$

    e. $L^ML^M \times L^NL^N$

15. *Assume that long ear lobes in humans are an autosomal dominant trait that exhibits 30% penetrance. A person who is heterozygous for long ear lobes mates with a person who is homozygous for normal ear lobes. What is the probability that their first child will have long ear lobes?

16. DATA ANALYSIS Club foot is one of the most common congenital skeletal abnormalities, with a worldwide incidence of about 1 in 1000 births. Both genetic and nongenetic factors are thought to be responsible for club foot. C. A. Gurnett et al. (2008. *American Journal of Human Genetics* 83:616–622) identified a family in which club foot was inherited as an autosomal dominant trait with incomplete penetrance. They discovered a mutation in the *PITXI* gene that caused club foot in this family. Through DNA testing, they determined that 11 people in the family carried the *PITXI*

mutation, but only 8 of these people had club foot. What is the penetrance of the *PITX1* mutation in this family?

17. *When a Chinese hamster with white spots is crossed with another hamster that has no spots, approximately $^1/_2$ of the offspring have white spots and $^1/_2$ have no spots. When two hamsters with white spots are crossed, $^2/_3$ of the offspring possess white spots and $^1/_3$ have no spots.

    a. What is the genetic basis of white spotting in Chinese hamsters?

    b. How might you go about producing Chinese hamsters that breed true for white spotting?

18.

In the early 1900s, Lucien Cuénot studied the genetic basis of yellow coat color in mice (discussed on p. 116). He carried out a number of crosses between two yellow mice and obtained what he thought was a 3:1 ratio of yellow to gray mice in the progeny. The following table gives Cuénot's actual results, along with the results of a much larger series of crosses carried out by Castle and Little (W. E. Castle and C. C. Little. 1910. *Science* 32:868–870).

Progeny resulting from crosses of yellow × yellow mice

Investigators	Yellow progeny	Non-yellow progeny	Total progeny
Cuénot	263	100	363

Castle and Little	800	435	1235
Both combined	1063	535	1598

a. Using a chi-square test, determine whether Cuénot's results are significantly different from the 3:1 ratio that he thought he observed. Are they different from a 2:1 ratio?

b. Determine whether Castle and Little's results are significantly different from a 3:1 ratio. Are they different from a 2:1 ratio?

c. Combine the results of Castle and Little and Cuénot and determine whether they are significantly different from a 3:1 ratio and a 2:1 ratio.

d. Offer an explanation for the different ratios that Cuénot and Castle and Little obtained.

19. *In the pearl-millet plant, color is determined by three alleles at a single locus: Rp^1 (red), Rp^2 (purple), and *rp* (green). Red is dominant to purple and green, and purple is dominant to green ($Rp^1 > Rp^2 > rp$). Give the expected phenotypes and ratios of offspring produced by the following crosses.

a. $Rp^1/Rp^2 \times Rp^1/rp$

b. $Rp^1/rp \times Rp^2/rp$

c. $Rp^1/Rp^2 \times Rp^1/Rp^2$

d. $Rp^2/Rp \times Rp/Rp$

e. $Rp/Rp \times Rp^1/Rp^2$

20. If there are five alleles at a locus, how many genotypes can there be at this locus? How many different kinds of homozygotes can there be? How many genotypes and homozygotes can there be with eight alleles at a locus?

21. Turkeys have black, bronze, or black-bronze plumage. Examine the results of the following crosses:

Parents	Offspring
Cross 1: black and bronze	all black
Cross 2: black and black	$^3/_4$ black, $^1/_4$ bronze
Cross 3: black-bronze and black-bronze	all black-bronze
Cross 4: black and bronze	$^1/_2$ black, $^1/_4$ bronze, $^1/_4$ black-bronze
Cross 5: bronze and black-bronze	$^1/_2$ bronze, $^1/_2$ black-bronze
Cross 6: bronze and bronze	

	$^3/_4$ bronze, $^1/_4$ black-bronze

Do you think these differences in plumage arise from incomplete dominance between two alleles at a single locus? If yes, support your conclusion by assigning symbols to each allele and providing genotypes for all turkeys in the crosses. If your answer is no, provide an alternative explanation and assign genotypes to all turkeys in the crosses.

22. In rabbits, an allelic series helps to determine coat color: *C* (full color), c^{ch} (chinchilla, gray color), c^{h} (Himalayan, white with black extremities), and *c* (albino, all-white). The *C* allele is dominant to all others, c^{ch} is dominant to c^{h} and *c*, c^{h} is dominant to *c*, and *c* is recessive to all the other alleles. This dominance hierarchy can be summarized as $C > c^{ch} > c^{h} > c$. The rabbits in the following list are crossed and produce the progeny shown. Give the genotypes of the parents for each cross:

Phenotypes of parents	Phenotypes of offspring
a. full color × albino	$^1/_2$ full color, $^1/_2$ albino
b. Himalayan × albino	$^1/_2$ Himalayan, $^1/_2$ albino
c. full color × albino	$^1/_2$ full color, $^1/_2$ chinchilla
d. full color × Himalayan	$^1/_2$ full color, $^1/_4$ Himalayan, $^1/_4$ albino

e. full color × full color	$^3/_4$ full color, $^1/_4$ albino

23. In this chapter, we considered Joan Barry's paternity suit against Charlie Chaplin and how, on the basis of blood types, Chaplin could not have been the father of her child.

    a. What blood types are possible for the father of Barry's child?

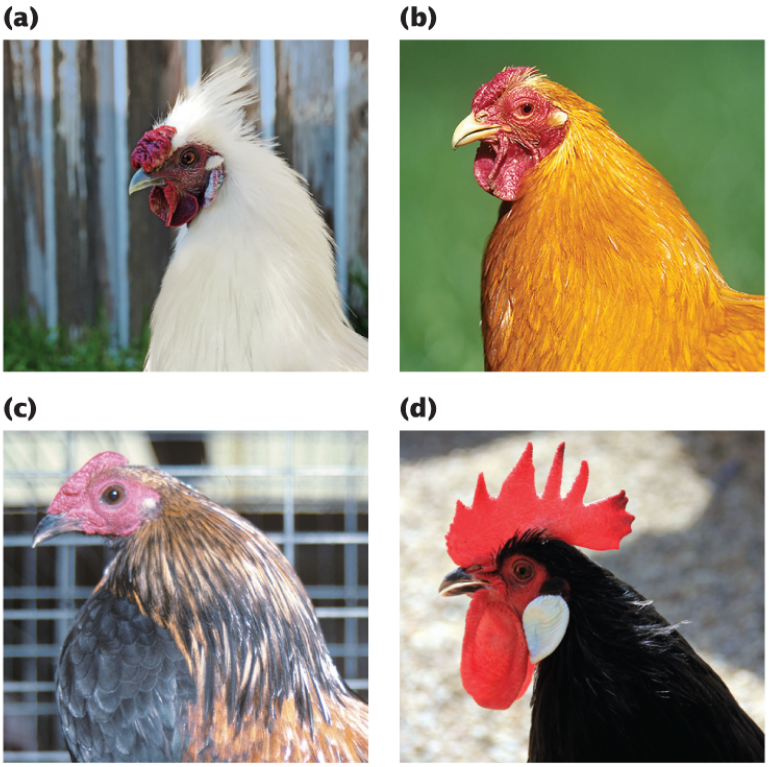
    b. If Chaplin had possessed one of these blood types, would that prove that he fathered Barry's child?

24. *A woman has blood type A M. She has a child with blood type AB MN. Which of the following blood types could *not* be that of the child's father? Explain your reasoning.

George	O	N
Tom	AB	MN
Bill	B	MN
Claude	A	N
Henry	AB	M

Section 5.2

25. *In chickens, comb shape is determined by alleles at two loci (*R*, *r* and *P*, *p*). A walnut comb is produced when at least one dominant allele *R* is present at one locus and at least one dominant allele *P* is present at a second locus (genotype *R*_ *P*_). A rose comb is produced when at least one dominant allele is present at the first locus and two recessive alleles are present at the second locus (genotype *R*_ *pp*). A pea comb is produced when two recessive alleles are present at the first locus and at least one dominant allele is present at the second (genotype *rr P*_). If two recessive alleles are present at the first and at the second loci (*rr pp*), a single comb is produced. Progeny with what types of combs and in what proportions will result from the following crosses?



Part a: ANGHI/Getty Images. Part b: Juniors Bildarchiv GmbH/ Alamy Stock Photo. Part c: Dapne Godfrey Trust/Animals Animals. Part d: FL collection/Alamy Stock Photo.

Comb shape: (a) walnut; (b) rose; (c) pea; (d) single.

a. *RR PP* × *rr pp*

b. *Rr Pp* × *rr pp*

c. *Rr Pp* × *Rr Pp*

d. *Rr pp* × *Rr pp*

e. *Rr pp* × *rr Pp*

f. *Rr pp* × *rr pp*

26.

*Tatuo Aida investigated the genetic basis of color variation in the medaka (*Aplocheilus latipes*), a small fish found in Japan (T. Aida. 1921. *Genetics* 6:554–573). Aida found that genes at two loci (*B*, *b* and *R*, *r*) determine the color of the fish: fish with a dominant allele at both loci (*B*_ *R*_) are brown, fish with a dominant allele at the *B* locus only (*B*_ *rr*) are blue, fish with a dominant allele at the *R* locus only (*bb R*_) are red, and fish with recessive alleles at both loci (*bb rr*) are white. Aida crossed a homozygous brown fish with a homozygous white fish. He then backcrossed the F_1 with the homozygous white parent and obtained 228 brown fish, 230 blue fish, 237 red fish, and 222 white fish.

a. Give the genotypes of the backcross progeny.

b. Use a chi-square test to compare the observed numbers of backcross progeny with the number expected. What conclusion can you make from your chi-square results?

c. What results would you expect for a cross between a homozygous red fish and a white fish?

d. What results would you expect if you crossed a homozygous red fish with a homozygous blue fish and then backcrossed the F_1 with a homozygous red parental fish?

27. A variety of opium poppy (*Papaver somniferum*) with lacerate leaves was crossed with a variety that has normal leaves. All the F_1 had lacerate leaves. Two F_1 plants were interbred to produce the F_2. Of the F_2, 249 had lacerate leaves and 16 had normal leaves. Give genotypes for all the plants in the P, F_1, and F_2 generations. Explain how lacerate leaves are determined in the opium poppy.

28.

E. W. Lindstrom crossed two corn plants with green seedlings and obtained the following progeny: 3583 green seedlings, 853 virescent-white seedlings, and 260 yellow seedlings (E. W. Lindstrom. 1921. *Genetics* 6:91–110).

a. Give the genotypes for the green, virescent-white, and yellow progeny.

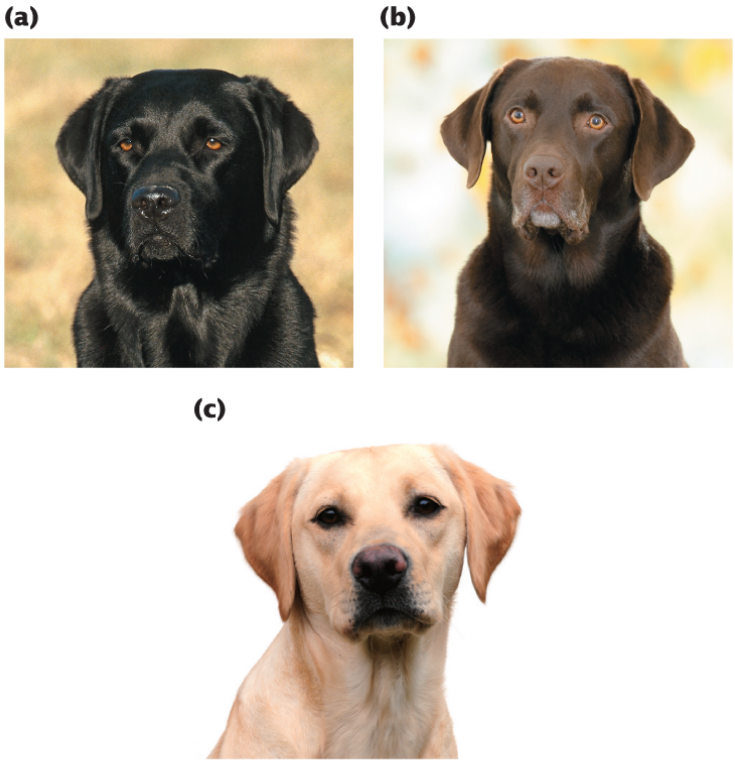
b. Explain how color is determined in these seedlings.

c. Is there epistasis among the genes that determine color in the corn seedlings? If so, which gene is epistatic and which is hypostatic?

29. *A dog breeder liked yellow and brown Labrador retrievers. In an attempt to produce yellow and brown puppies, he mated a yellow Labrador male and a brown Labrador female. Unfortunately, all the puppies produced in this cross were black. (See pp. 126–128 for a discussion of the genetic basis of coat color in Labrador retrievers.)

a. Explain this result.

b. How might the breeder go about producing yellow and brown Labradors?



Parts a and b: Juniors Bildarchiv/Alamy.
Part c: c.byatt-norman/Shutterstock.

Coat color in Labrador retrievers: (a) black; (b) brown; (c) yellow.

30. When a yellow female Labrador retriever was mated with a brown male, half of the puppies were brown and half were yellow. The same female, when mated with a different brown male, produced only brown offspring. Explain these results.

31. *A summer-squash plant that produces disc-shaped fruit is crossed with a summer-squash plant that produces long fruit. All the F_1 have disc-shaped fruit. When the F_1 are intercrossed, F_2 progeny are produced in the following ratio: $^9/_{16}$ disc-shaped fruit : $^6/_{16}$ spherical fruit : $^1/_{16}$ long fruit. Give the genotypes of the F_2 progeny.

32. Some sweet-pea plants have purple flowers and others have white flowers. A homozygous variety of sweet pea that has purple flowers is crossed with a homozygous variety that has white flowers. All the F_1 have purple flowers. When these F_1 self-fertilize, the F_2 appear in a ratio of $^9/_{16}$ purple to $^7/_{16}$ white.

    a. Give genotypes for the purple and white flowers in these crosses.

    b. Draw a hypothetical biochemical pathway to explain the production of purple and white flowers in sweet peas.

33. *Refer to the discussion of how coat color and pattern are determined in dogs.

    a. Why are Irish setters not black in color?

    b. Can a poodle crossed with any other breed produce spotted puppies? Why or why not?

c. If a St. Bernard is crossed with a Doberman, what will be the coat color of the offspring: solid, yellow, saddle, or bicolor?

d. If a Rottweiler is crossed with a Labrador retriever, what will be the coat color of the offspring: solid, yellow, saddle, or bicolor?

Section 5.3

34. Male-limited precocious puberty results from a rare, sex-limited autosomal allele (*P*) that is dominant to the allele for normal puberty (*p*) and is expressed only in males. Bill underwent precocious puberty, but his brother Jack and his sister Beth underwent puberty at the usual time, between the ages of 10 and 14. Although Bill's mother and father underwent normal puberty, two of his maternal uncles (his mother's brothers) underwent precocious puberty. All of Bill's grandparents underwent normal puberty. Give the most likely genotypes for all the relatives mentioned in this family.

35. *In some goats, the presence of horns is produced by an autosomal gene that is dominant in males and recessive in females. A horned female is crossed with a hornless male. The F_1 offspring are intercrossed to produce the F_2. What proportion of the F_2 females will have horns?

36. In goats, a beard is produced by an autosomal allele that is dominant in males and recessive in females. We'll use the symbol B^b for the beard allele and B^+ for the beardless allele. Another independently assorting autosomal allele that produces a black coat (*W*) is dominant to the allele for white coat (*w*). Give the

phenotypes and their expected proportions for the following crosses.

a. B^+B^b *Ww male* × B^+B^b *Ww female*

b. B^+B^b *Ww male* × B^+B^b *Ww female*

c. B^+B^+ *Ww male* × B^bB^b *Ww female*

d. B^+B^b *Ww male* × B^bB^b *Ww female*

37. Cock feathering in chickens is an autosomal recessive trait that is limited to males. List all possible genotypes for the chicken shown in

a. **Figure 5.13a**

b. **Figure 5.13b**

c. **Figure 5.13c**

38. J. K. Breitenbecher (1921. *Genetics* 6:65–86) investigated the genetic basis of color variation in the four-spotted cowpea weevil (*Bruchus quadrimaculatus*). The weevils were red, black, white, or tan. Breitenbecher found that four alleles (R, R^b, R^w, and r) at a single locus determine color. The alleles exhibit a dominance hierarchy, with red (R) dominant to all other alleles, black (R^b) dominant to white (R^w) and tan (r), white dominant to tan, and tan recessive to all others ($R \rightarrow R^b \rightarrow R^w \rightarrow r$). The following genotypes encode each of the colors:

RR, RR^b, RR^w, Rr	red

R^bR^b, R^bR^w, R^br	black
R^wR^w, R^wr	white
Rr	tan

Color variation in this species is limited to females: males carry color genes but are always tan regardless of their genotype. For each of the following crosses carried out by Breitenbecher, give all possible genotypes of the parents.

Parents	Progeny
a. tan ♀ × tan ♂	78 red ♀, 70 white ♀, 184 tan ♂
b. black ♀ × tan ♂	151 red ♀, 49 black ♀, 61 tan ♀, 249 tan ♂
c. white ♀× tan ♂	32 red ♀, 31 tan ♂
d. black ♀ × tan ♂	3586 black ♀, 1282 tan ♀, 4791 tan ♂
e. white ♀ × tan ♂	594 white ♀, 189 tan ♀, 862 tan ♂
f. black ♀ ×	88 black ♀, 88 tan ♀, 186 tan ♂

tan ♂	
g. tan ♀ × tan ♂	47 white ♀, 51 tan ♀, 100 tan ♂
h. red ♀ × tan ♂	1932 red ♀, 592 tan ♀, 2587 tan ♂
i. white ♀ × tan ♂	13 red ♀, 6 white ♀, 5 tan ♀, 19 tan ♂
j. red ♀ × tan ♂	190 red ♀, 196 black ♀, 311 tan ♂
k. black ♀ × tan ♂	1412 black ♀, 502 white ♀, 1766 tan ♂

39. *The direction of shell coiling in the snail *Lymnaea peregra* (discussed in the introduction to this chapter) results from genetic maternal effect. An autosomal allele for a right-handed, or dextral, shell (s^+) is dominant to the allele for a left-handed, or sinistral, shell (s). A pet snail called Martha is sinistral and reproduces only as a female (the snails are hermaphroditic). Indicate which of the following statements are true and which are false. Explain your reasoning in each case.

a. Martha's genotype *must* be *ss*.

b. Martha's genotype *cannot* be s^+s^+.

c. All the offspring produced by Martha *must* be sinistral.

d. At least some of the offspring produced by Martha *must* be sinistral.

e. Martha's mother *must* have been sinistral.

f. All of Martha's brothers *must* be sinistral.

40. If the F_2 dextral snails with genotype s^+s in **Figure 5.17** undergo self-fertilization, what phenotypes and proportions are expected to occur in the progeny?

41. Hypospadias, a birth defect in human males in which the urethra opens on the shaft instead of at the tip of the penis, results from an autosomal dominant gene in some families. Females who carry the gene show no effects. Is this birth defect an example of an X-linked trait, a Y-linked trait, a sex-limited trait, a sex-influenced trait, or genetic maternal effect? Explain your answer.

42. In unicorns, two autosomal loci interact to determine the type of tail. One locus controls whether a tail is present at all; the allele for a tail (*T*) is dominant to the allele for tailless (*t*). If a unicorn has a tail, then alleles at a second locus determine whether the tail is curly or straight. Farmer Baldridge has two unicorns with curly tails: when he crosses them, $^1/_2$ of the progeny have curly tails, $^1/_4$ have straight tails, and $^1/_4$ do not have a tail. Give the genotypes of the parents and progeny in Farmer Baldridge's cross. Explain how he obtained the 2:1:1 phenotypic ratio in his cross.

43. In 1983, a sheep farmer in Oklahoma noticed in his flock a ram that possessed increased muscle mass in his hindquarters. Many of the offspring of this ram possessed the same trait, which

became known as the callipyge phenotype (*callipyge* is Greek for "beautiful buttocks"). The mutation that caused the callipyge phenotype was eventually mapped to a position on the sheep chromosome 18.

When the male callipyge offspring of the original mutant ram were crossed with normal females, they produced the following progeny: $^1/_4$ male callipyge, $^1/_4$ female callipyge, $^1/_4$ male normal, and $^1/_4$ female normal. When the female callipyge offspring of the original mutant ram were crossed with normal males, all the offspring were normal. Analysis of the chromosomes of these offspring of callipyge females showed that half of them received a chromosome 18 with the allele encoding callipyge from their mother. Propose an explanation for the inheritance of the allele for callipyge. How might you test your explanation?

Section 5.5

44. Which of the following statements describes an example of a phenocopy? Explain your reasoning.

    a. Phenylketonuria results from a recessive mutation that causes light skin as well as intellectual disability.

    b. Human height is influenced by genes at many different loci.

    c. Dwarf plants and mottled leaves in tomatoes are caused by separate genes that are linked.

    d. Vestigial wings in *Drosophila* are produced by a recessive mutation. This trait is also produced by high temperature

during development.

e. Intelligence in humans is influenced by both genetic and environmental factors.

45. *Long ears in some dogs are an autosomal dominant trait. Two dogs mate and produce a litter in which 75% of the puppies have long ears. Of the dogs with long ears in this litter, $^1/_3$ are known to be phenocopies. What are the most likely genotypes of the two parents of this litter?

46.

White eyes is an X-linked recessive mutation in *Drosophila* that results in eyes that are devoid of the normal red pigment seen in wild-type flies (see Chapter 4). The white locus encodes an ABC transporter protein that, when functional, moves a variety of substances across the cell membrane including pigment precursors. When the transporter protein is defective due to a mutation at the white locus, the precursors are not present inside the cell and no eye pigments are produced. Mutations at the white locus also affect mating behavior and the fly's ability to recover from oxygen deprivation.

a. What phenomenon is illustrated by the different phenotypic effects of mutations at the white locus?

b. Propose an explanation for why mutations at the white eye locus have such differing effects as eye color, mating behavior, and physiology.

47. The fly with vestigial wings shown in the lower-left corner of Figure 5.19 is crossed with the fly with normal wings shown in the upper-right corner of the figure. If the progeny are reared at 31°C, what percentage will have vestigial wings?

CHALLENGE QUESTIONS

Section 5.1

48. DATA ANALYSIS Pigeons have long been the subject of genetic studies. Indeed, Charles Darwin bred pigeons in the hope of unraveling the principles of heredity but was unsuccessful. A series of genetic investigations in the early 1900s worked out the hereditary basis of color variation in these birds. W. R. Horlancher was interested in the genetic basis of kiteness, a color pattern that consists of a mixture of red and black stippling of the feathers. He carried out the following crosses to investigate the genetic relation of kiteness to black and red feather color (W. R. Horlancher. 1930. *Genetics* 15:312–346).

Cross	Offspring
kitey × kitey	16 kitey, 5 black, 3 red
kitey × black	6 kitey, 7 black
red × kitey	18 red, 9 kitey, 6 black

a. On the basis of these results, propose a hypothesis to explain the inheritance of kitey, black, and red feather color in pigeons. (Hint: Assume that two loci are involved and some type of epistasis occurs.)

b. For each of the preceding crosses, test your hypothesis by using a chi-square test.

Section 5.3

49. Suppose that you are tending a mouse colony at a genetic research institute, and one day you discover a mouse with twisted ears. You breed this mouse with twisted ears and find that the trait is inherited. Both male and female mice may have twisted ears, but when you cross a twisted-eared male with a normal-eared female, you obtain results that differ from those obtained when you cross a twisted-eared female with a normal-eared male: the reciprocal crosses give different results. Describe how you would determine whether this trait results from a sex-linked gene, a sex-influenced gene, genetic maternal effect, a cytoplasmically inherited gene, or genomic imprinting. What crosses would you conduct, and what results would be expected with these different types of inheritance?

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 5.1

1. Over a thousand different alleles at the *CFTR* locus have been discovered that can cause cystic fibrosis. What difficulties might the presence of so many different alleles at this locus create for the diagnosis and treatment of cystic fibrosis?

Section 5.2

2. Could you carry out a complementation test on two dominant mutations? Why or why not?

Section 5.3

3. In goats, a beard is produced by an autosomal allele that is dominant in males and recessive in females. Is it possible to cross two bearded goats and obtain a beardless male offspring? Why or why not? What about a bearded female offspring?

4. In chickens, cock feathering is an autosomal recessive trait that is limited to males. Is it possible to cross a cock-feathered male with a hen-feathered female and get male progeny that are cock-feathered? Explain your reasoning.

5. Suppose you observed a new mutant phenotype, notched ears, that appears only in male mice. How might you go about determining whether notched ears is a Y-linked trait or a sex-limited trait? What crosses would you carry out to distinguish between these two modes of inheritance?

6. Phenylketonuria (PKU) is an autosomal recessive disease that results from a defect in an enzyme that normally metabolizes the amino acid phenylalanine; when this enzyme is defective, high

levels of phenylalanine cause brain damage. In the past, most children with PKU became intellectually disabled. Fortunately, intellectual disability can be prevented in these children by carefully controlling the amount of phenylalanine in the diet. The diet is usually applied during childhood, when brain development is taking place. As a result of this treatment, many people with PKU now reach reproductive age. Children born to women with PKU (who are no longer on a phenylalanine-restricted diet) frequently have low birth weight, developmental abnormalities, and intellectual disabilities. However, children of men with PKU do not have these problems.

a. Provide an explanation for these observations.

b. What type of genetic effect is this? Explain your reasoning.

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Explain how the direction of coiling in *Lymnaea peregra* is determined by creating a concept map that includes the following terms: *Lymnaea peregra*, outcrossing, shell coiling, genetic maternal effect, dextral, sinistral, dominant allele, self-fertilization, genotype of maternal parent, recessive allele.

Section 5.1

2. Create a concept map that outlines the different types of dominance and their relationships.

Section 5.3

3. Create a concept map illustrating the ways that sex may influence heredity. Include the following terms in your map (you may add additional terms): influences of sex on heredity, sex-influenced characteristic, cytoplasmic inheritance, genomic imprinting, sex-linked characteristic, sex-limited characteristic, genetic maternal effect, sex chromosomes, autosomes, organelle DNA.

Section 5.5

4. With a concept map, depict the relationships among the following terms: characteristics, discontinuous characteristics, phenocopy, continuous characteristics, quantitative characteristics, polygenic characteristics, many genes, environmental factors.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 6

Pedigree Analysis, Applications, and Genetic Testing

Phanie/Science Source.

Fingerprints are unique to each person. A few people have a condition known as adermatoglyphia (ADG), in which fingerprints are completely absent; this condition is inherited as an autosomal dominant trait. Shown here is a human fingerprint superimposed on DNA sequence information.

The Mystery of the Missing Fingerprints

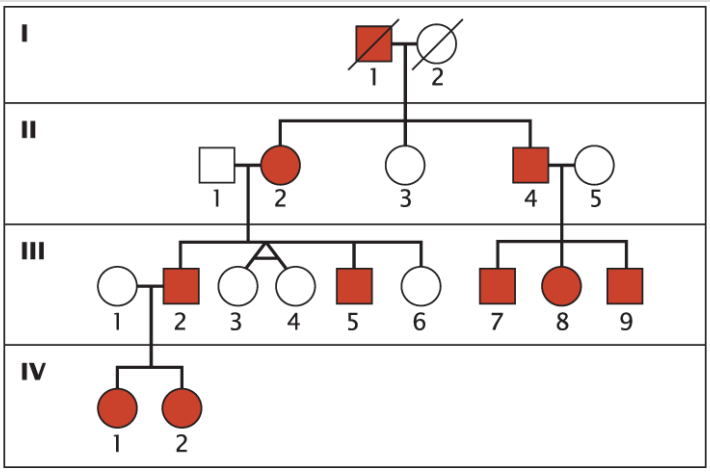
In 2007, a 29-year-old Swiss woman attempted to enter the United States. Although her appearance matched the photograph on her passport, she failed a fingerprint check, not because her fingerprints matched those of a terrorist, but rather because she had no fingerprints at all—her fingers were completely devoid of any prints. She was delayed for several hours as puzzled immigration officials tried to decide what to do about a person with no fingerprints.

Fingerprints are among our most unique and permanent traits. No two individuals, not even identical twins, share the same fingerprints. Fingerprints are technically termed *epidermal ridges* or *dermatoglyphic patterns*; these patterns are found on our fingers, toes, palms, and the soles of our feet. Epidermal ridges appear long before birth—they are fully formed by the 17th week of pregnancy—and are permanent for life. Research shows that fingerprint patterns are clearly influenced by heredity, but random factors also play a role. One of the first scientists to study fingerprints was Francis Galton, a cousin of Charles Darwin. In the late 1800s, Galton established that no two individuals have the same fingerprints and showed that fingerprints of relatives are more similar than those of unrelated people.

The complete absence of fingerprints, as exhibited by the Swiss woman at the airport, is an extremely rare condition known as adermatoglyphia (ADG). Dubbed "immigration delay disease" because of the hassle it creates when people with the condition attempt to cross borders, ADG has been documented in only a few people from four families around the world. In ADG, fingerprints are absent at birth and never develop. Otherwise, the disorder produces no harmful effects.

In 2011, geneticists in Israel and Switzerland solved the mystery of missing fingerprints in people with ADG. Janna Nousbeck and her colleagues examined the condition in a large Swiss family in which some members had normal fingerprints and other members were missing them entirely (**Figure 6.1**). In this family, ADG exhibits the hallmark characteristics of an autosomal dominant trait. The researchers took blood samples from family members who lacked fingerprints and from those with normal fingerprints. They extracted DNA from the blood and genotyped the family members for 6000 single-nucleotide polymorphisms (SNPs), which are DNA sequences that vary in a single nucleotide. By comparing the SNPs of family members with and without fingerprints, they were able to determine that the gene for ADG was located in a specific interval on the long arm of chromosome 4. One of the genes in this region is *SMARCAD1*, which encodes a short form of a protein found exclusively in the skin. Sequencing of the gene revealed that family members with ADG possessed a mutation not found in those with fingerprints. The mutation causes abnormal splicing in RNA transcribed from the

gene, with the result that the RNA is less stable. How the decreased stability of this RNA leads to ADG is not known, but scientists hope that the identification of this gene will lead to a better understanding of how fingerprints develop.



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6.1 Pedigree of Swiss family with adermatoglyphia (absence of fingerprints). Squares represent males; circles represent females. Colored squares and circles are people with adermatoglyphia.

Description

Generation 1 has a couple with the male positive for adermatoglyphia. Both the male and female in generation 1 are crossed out. Generation 2 has 1 female with the condition, one female without the condition, and one male with the condition. Both of the siblings with adermatoglyphia mate with unaffected people and have offspring. In generation 3, the positive female from generation 2 has 2 male offspring with the condition, a set of twin females without the condition, and 1 female without the condition. From these siblings, one of the positive males mates with a female and both of their female offspring in generation 4 are positive for adermatoglyphia. The positive male from generation 2 has 2 male offspring and 1 female offspring in generation 3 and all of them have adermatoglyphia.

THINK-PAIR-SHARE

- If identical twins share 100% of the same genes, why do they have different fingerprints?
- What characteristics of the pedigree in Figure 6.1 suggest that adermatoglyphia in this family is inherited as an autosomal dominant trait?

The absence of fingerprints is just one of a large number of human traits and diseases that are currently the focus of intensive genetic research. In this chapter, we consider human genetic characteristics and examine three important techniques used by geneticists to investigate these characteristics: pedigrees, twin studies, and adoption studies. At the end of the chapter, we see how the information garnered with these techniques can be used in genetic counseling and prenatal diagnosis.

Keep in mind as you read this chapter that many important characteristics are influenced by both genes and environment, and that separating these factors in humans is always difficult. Studies of twins and adopted people are designed to distinguish the effects of genes and environment, but such studies are based on assumptions that may be difficult to meet for some human characteristics, particularly behavioral ones. Therefore, it's always prudent to interpret the results of such studies with caution.

6.1 The Study of Genetics in Humans Is Constrained by Special Features of Human Biology and Culture

Humans are both the best and the worst of all organisms for genetic study. On the one hand, we know more about human anatomy, physiology, and biochemistry than about that of most other organisms, so many well-characterized traits are available for study. Families often keep detailed records about their members extending back many generations. Furthermore, a number of important human diseases have a genetic component, so the incentive for understanding human inheritance is tremendous. On the other hand, the study of human genetic characteristics presents some major challenges.

First, controlled matings are not possible. With other organisms, geneticists carry out specific crosses to test their hypotheses about inheritance. We have seen, for example, that the testcross provides a convenient way to determine whether an individual with a dominant trait is homozygous or heterozygous. Unfortunately (for the geneticist at least), matings between humans are usually determined by romance, family expectations, or—occasionally—accident rather than by the requirements of science.

Another challenge is that humans have a long generation time. Human reproductive age is not normally reached until 10 to 14 years after birth, and most people do not reproduce until they are 18 years of age or older; thus, generation time in humans is usually about 20 years. This long generation time means that even if geneticists could control human crosses, they would have to wait an average of 40 years just to observe the F_2 progeny. In contrast, generation time in *Drosophila* is 2 weeks; in bacteria, it's a mere 20 minutes.

Finally, human family size is generally small. Observation of even the simple genetic ratios that we learned in Chapter 3 would require a substantial number of progeny in each family. When parents produce only 2 children, the detection of a 3:1 ratio is impossible. Even an extremely large family of 10 to 15 children would not permit the recognition of a dihybrid 9 : 3 : 3 : 1 ratio.

Although these special constraints make genetic studies of humans more complex, understanding human heredity is tremendously important. Therefore, geneticists have been forced to develop techniques that are uniquely suited to human biology and culture. ❯

TRY PROBLEM 18

THINK-PAIR-SHARE Question 1

CONCEPTS

The study of human inheritance is constrained by the inability to control genetic crosses, a long generation time, and a small number of offspring.

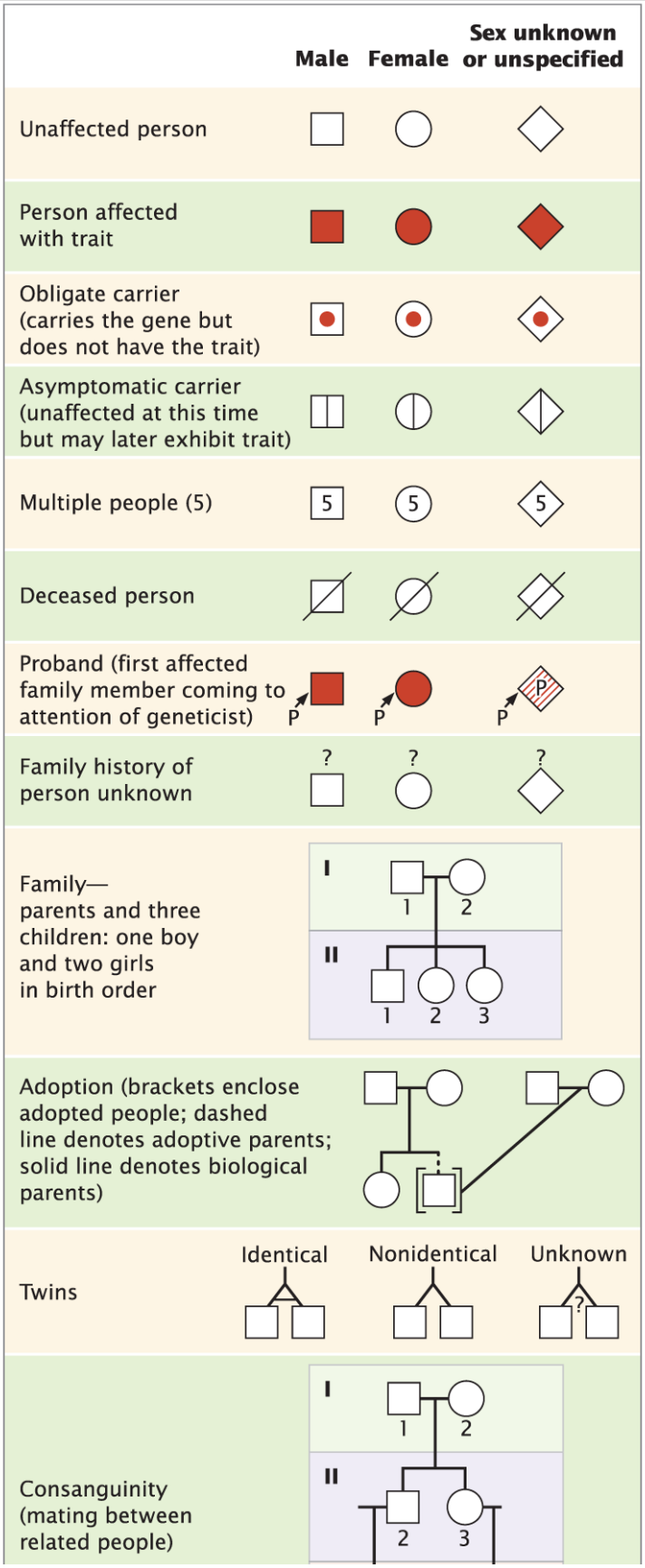
6.2 Geneticists Often Use Pedigrees to Study the Inheritance of Characteristics in Humans

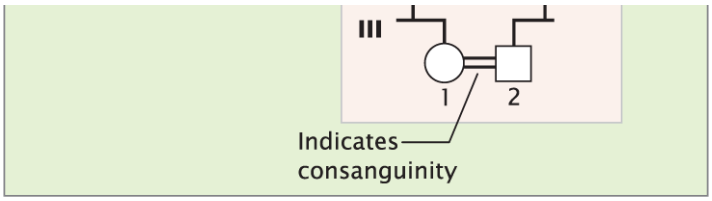
An important technique used by geneticists to study human inheritance is the analysis of pedigrees. A **pedigree** is a pictorial representation of a family history, essentially a family tree that outlines the inheritance of one or more characteristics. When a particular characteristic or disease is observed in a person, a geneticist often studies the family of that affected person by drawing a pedigree. Today, computer programs are often used to create and analyze pedigrees. These programs may calculate a variety of genetic parameters from pedigree, including measures of relatedness, inbreeding coefficients (see Chapter 25), genotype predictions, genetic risk values, and estimates of linkage among genes (see Chapter 7).

Symbols Used in Pedigrees

The symbols commonly used in pedigrees are summarized in **Figure 6.2**. Males in a pedigree are represented by squares, females by circles. A horizontal line drawn between two symbols representing a man and a woman indicates a mating; children are connected to their parents by vertical lines extending downward from the parents.

The pedigree shown in **Figure 6.3a** illustrates a family with Waardenburg syndrome, an autosomal dominant type of deafness that may be accompanied by fair skin, a white forelock, and visual problems (**Figure 6.3b**). People who exhibit the trait of interest are represented by filled circles and squares; in the pedigree of Figure 6.3a, the filled symbols represent members of the family who have Waardenburg syndrome. Unaffected members are represented by open circles and squares. The person from whom the pedigree is initiated is called the **proband** and is usually designated by the letter *P* and an arrow (IV-2 in Figure 6.3a).


Male
Female
Sex unknown or unspecified
Unaffected person
Person affected with trait
Obligate carrier (carries the gene but does not have the trait)
Asymptomatic carrier (unaffected at this time but may later exhibit trait)
Multiple people (5)
5
Deceased person
Proband (first affected family member coming to attention of geneticist)
P
Family history of person unknown
?
Family—parents and three children: one boy and two girls in birth order
I
1
2
II
1
2
3
Adoption (brackets enclose adopted people; dashed line denotes adoptive parents; solid line denotes biological parents)
Twins
Identical
Nonidentical
Unknown
Consanguinity (mating between related people)



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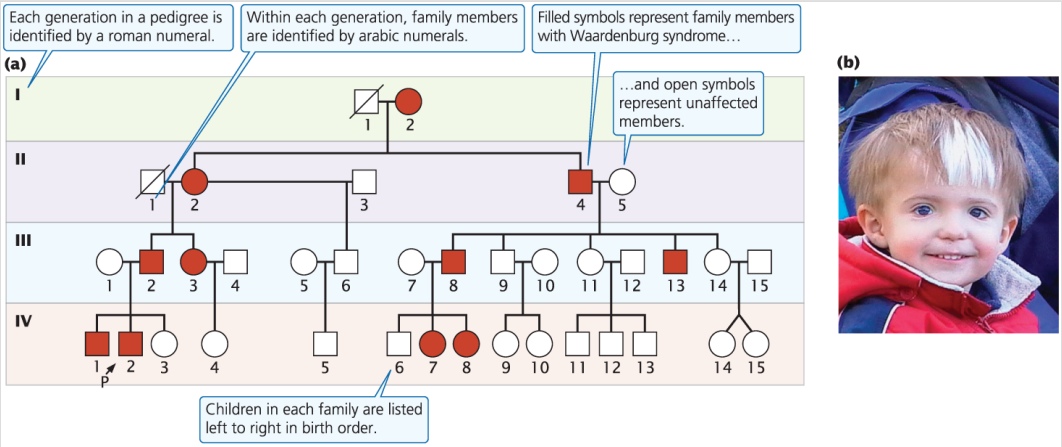
6.2 Standard symbols are used in pedigrees.

Description

In a pedigree, a male is represented by a square, a female by a circle, and a person of unknown sex by a diamond. Unaffected individuals have uncolored shapes. For affected persons, the shape is filled in with red. Obligate carriers that carry the gene but do not display the trait are represented by the shape with a smaller colored circle inside. Asymptomatic carriers are those who are unaffected at this time but may later exhibit trait have a vertical line drawn in the center of the symbols. Multiple persons may be indicated by a number inside the shape rather than multiple shapes. A deceased person will have the shape crossed out with a slash. The proband, or the first affected family member coming to the attention of the geneticist, is indicated with the solid color and a capital P with an arrow pointing to the shape from a left side. A person of unknown sex who is proband may have multiple lines inside the shape rather than it being completely filled in. When a person has an unknown family history, there is a question mark over their corresponding symbol. Each generation is indicated by a roman numeral and each person in a generation is numbered. In a family with parents and three children out of which one is boy and two girls in birth order, a pedigree chart is drawn is as follows: Two blocks are present. One has Roman numeral 1 written on left and has a square and a circle connected by a horizontal line in between. From the line, a perpendicular line is drawn downwards to another block with roman numeral 2 written on left. The line divides into 3 branches that have labeled shapes at the end as follows: 1 square, 2 circle, and 3 circle. Adoption is denoted by square brackets enclosing the symbol. Dashed line represents adoptive parents and solid line represents biological parents. The lines between twins are angled downward and triangular in shape. For identical twins, an extra line is drawn between the lines, resulting in a shape that looks like an big A. Unknown twin status is designated with a question mark below the upside down V. Consanguinity or mating between related people is indicated by two parallel vertical lines drawn between the symbols.



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Part b: Courtesy of Guy Rowland.

6.3 Pedigree of a person with Waardenburg syndrome. The proband (P) is the person from whom this pedigree is initiated. (a) Waardenburg syndrome is inherited as an autosomal dominant trait. (b) The syndrome is characterized by deafness, fair skin, visual problems, and a white forelock.

Description

Part “a” shows a pedigree chart. The pedigree chart a shows a couple in generation 1 where male is crossed out and the female is affected. A caption reads, “Each generation in a pedigree is identified by a Roman numeral.” The couple from generation 1 has one affected son and one affected daughter in the generation 2. A caption reads, “Within each generation, family members are identified by Arabic numerals.” The affected female in generation 2 mates with an unaffected male who is crossed out and has one affected son and affected female in the generation 3. The affected female from generation 2 also mates with another unaffected male and has an unaffected son in generation 3. The affected male from generation 2 mates with an unaffected female and has 2 affected sons, 1 unaffected son, and 2 unaffected daughters in generation 3. The affected male and female who were children of affected female and unaffected crossed out male from generation 2 mate with an unaffected female and an unaffected male respectively. The male has 2 affected sons and an unaffected daughter in generation 4. One of these sons is a proband. The female has 1 unaffected daughter in generation 4. The unaffected son in generation 3 who is child of affected female from generation 2 and an unaffected man, mates with an unaffected female and has one unaffected son in generation 4. Affected son of affected male from generation 2, mates with unaffected female and has 2 affected daughters and 1

unaffected son in generation 4. A caption reads, “Children in each family are listed left to right in birth order.” Unaffected son of affected male from generation 2, mates with an unaffected female and has two unaffected daughters in generation 4. An unaffected daughter of affected male from generation 2, mates with an unaffected male and has 3 unaffected sons. Another unaffected daughter of affected male from generation 2, mates with an unaffected male and has non-identical unaffected daughters in generation 4. Part “b” shows a photo of a child with a white forelock due to Waardenburg syndrome.

Let’s look closely at Figure 6.3 and consider some additional features of a pedigree. Each generation in a pedigree is identified by a roman numeral; within each generation, family members are assigned arabic numerals, and children in each family are listed in birth order from left to right. Person II-4, a man with Waardenburg syndrome, mated with II-5, an unaffected woman, and they produced five children. The oldest of their children is III-8, a male with Waardenburg syndrome, and the youngest is III-14, an unaffected female. ❯ **TRY** **PROBLEM 19a**

Analysis of Pedigrees

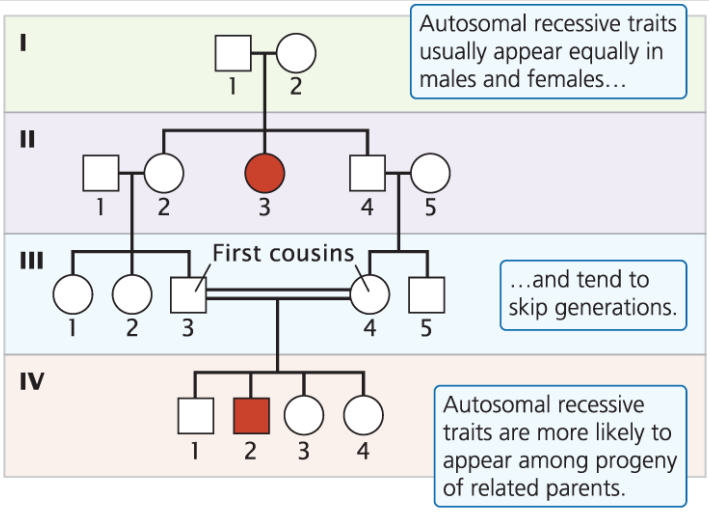
The limited number of offspring in most human families means that clear Mendelian ratios are usually impossible to discern in a single pedigree. Pedigree analysis requires a certain amount of genetic sleuthing, based on recognizing patterns associated with different modes of inheritance. For example, autosomal dominant traits should appear with equal frequency in both sexes and should not skip generations, provided that the trait is fully penetrant (see

Penetrance and Expressivity in Chapter 5) and not sex-influenced (see Sex-Influenced and Sex-Limited Characteristics in Chapter 5).

Certain patterns may exclude the possibility of a particular mode of inheritance. For instance, a son inherits his X chromosome from his mother. If we observe that a trait is passed from father to son, we can exclude the possibility of X-linked inheritance. In the following sections, the traits discussed are assumed to be fully penetrant and rare.

Autosomal Recessive Traits

Autosomal recessive traits normally appear with equal frequency in both sexes (unless penetrance differs in males and females) and appear only when a person inherits two alleles for the trait, one from each parent. If the trait is uncommon, most parents of affected offspring are heterozygous and unaffected; consequently, the trait seems to skip generations (**Figure 6.4**). Frequently, a recessive allele may be passed on for a number of generations without the trait appearing in a pedigree. When both parents are heterozygous, approximately one-fourth of the offspring are expected to express the trait, but this ratio will not be obvious unless the family is large. In the rare event that both parents are affected by an autosomal recessive trait, all the offspring will be affected.



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6.4 Autosomal recessive traits normally appear with equal frequency in both sexes and often skip generations.

Description

Generation 1 shows an unaffected couple. A caption reads, “Autosomal recessive traits usually appear equally in males and females and tend to skip generations.” The couple has 1 unaffected daughter, 1 affected daughter, and 1 unaffected son in generation 2. The unaffected female from generation 2 mates with an unaffected male and has 2 unaffected daughters and 1 unaffected son in generation 3. The unaffected male from generation 2 mates with an unaffected female and has 1 unaffected daughter and 1 unaffected son in generation 3. The unaffected son of unaffected female from generation 2 mates with the unaffected daughter of unaffected male from generation 2. As they are first cousins, it is a case of consanguinity. They have 2 unaffected daughters, 1 affected son, and 1 unaffected son in generation 4. A caption reads, “Autosomal recessive traits are more likely to appear among progeny of related parents.”

When a recessive trait is rare, most people outside affected families are homozygous for the normal allele. Thus, when an affected person mates with someone outside the family ($aa \times AA$), usually none of the children display the trait, although all will be carriers

(i.e., heterozygous). A recessive trait is more likely to appear in a pedigree when two people within the same family mate, because there is a greater chance of both parents carrying the same recessive allele. Mating between closely related people is called **consanguinity**. In the pedigree shown in Figure 6.4, individuals III-3 and III-4 are first cousins, and both are heterozygous for the recessive allele; when two heterozygotes mate, one-fourth of their children are expected to have the recessive trait.

CONCEPTS

Autosomal recessive traits appear with equal frequency in males and females. Affected children are commonly born to unaffected parents who are carriers of the gene for the trait, and the trait tends to skip generations. Recessive traits appear more frequently among the offspring of consanguineous matings.

✔ CONCEPT CHECK 1

Autosomal recessive traits often appear in pedigrees in which there have been consanguineous matings because these traits

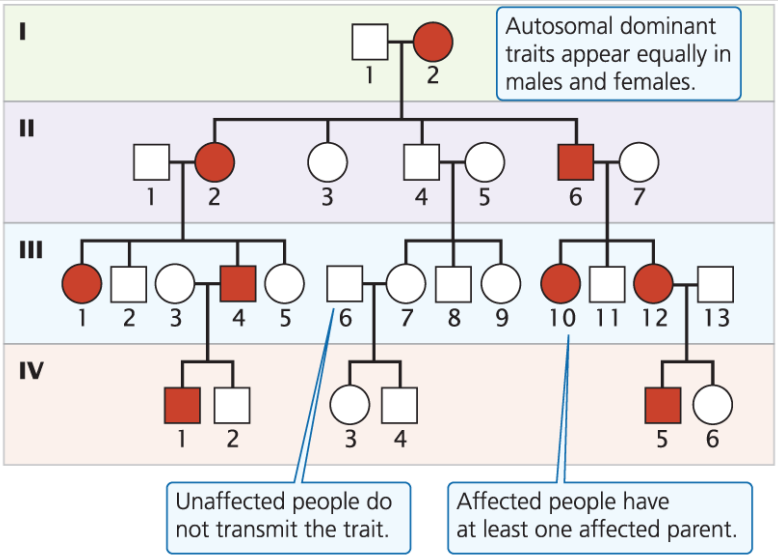
a. tend to skip generations.
b. appear only when both parents carry a copy of the gene for the trait, which is more likely when the parents are related.
c. usually arise in children born to parents who are unaffected.
d. appear equally in males and females.

A number of human metabolic diseases are inherited as autosomal recessive traits. One of them is Tay–Sachs disease. Children with Tay–Sachs disease appear healthy at birth but become listless and

weak at about 6 months of age. Gradually, their physical and neurological conditions worsen, leading to blindness, deafness, and eventually death at two to three years of age. The disease results from the accumulation of a lipid called G_{M2} ganglioside in the brain. A normal component of brain cells, G_{M2} ganglioside is usually broken down by an enzyme called hexosaminidase A, but children with Tay–Sachs disease lack this enzyme. Excessive G_{M2} ganglioside accumulates in the brain, causing swelling and, ultimately neurological symptoms. Heterozygotes have only one normal copy of the allele encoding hexosaminidase A and produce only about half the normal amount of the enzyme. However, this amount is enough to ensure that G_{M2} ganglioside is broken down normally, and heterozygotes are usually healthy.

Autosomal Dominant Traits

Autosomal dominant traits appear in both sexes with equal frequency, and both sexes are capable of transmitting these traits to their offspring. Every person with a dominant trait must have inherited the allele from at least one parent; autosomal dominant traits therefore do not skip generations (**Figure 6.5**). Exceptions to this rule arise when people acquire the trait as a result of a new mutation or when the trait has incomplete penetrance.



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6.5 Autosomal dominant traits normally appear with equal frequency in both sexes and do not skip generations.

Description

Generation 1 has a couple where the male is unaffected and the female is affected. Text reads, “Autosomal dominant traits appear equally in males and females.” They have 1 affected daughter, 1 unaffected daughter, 1 affected son, and 1 unaffected son in generation 2. The affected female from generation 2 mates with an unaffected male and has 1 affected son, 1 unaffected son, 1 affected daughter, and 1 unaffected daughter in generation 3. An unaffected male from generation 2 mates with an unaffected female and has 2 unaffected daughters and 1 unaffected son in generation 3. The affected male from generation 2 mates with an unaffected female and has 2 affected daughters and an unaffected son in generation 3. A caption reads, “Affected people have at least one affected parent.” The affected son of affected female from generation 2 mates with an unaffected female and has 1 affected son and 1 unaffected son in generation 4. An unaffected daughter of the unaffected male from generation 2 mates with an unaffected male and has 1 unaffected daughter and 1 unaffected son in generation 4. A caption reads, “Unaffected people do not transmit the trait.” An affected daughter of the affected male from generation 2 mates with an unaffected male and has 1 affected son and 1 unaffected daughter in generation 4.

If an autosomal dominant allele is rare, most people displaying the trait are heterozygous. When one parent is affected and heterozygous and the other parent is unaffected, approximately half of the offspring will be affected. If both parents have the trait and are heterozygous, approximately three-fourths of the children will be affected. Unaffected people do not transmit the trait to their descendants, provided that the trait is fully penetrant. In Figure 6.5, we see that none of the descendants of II-4 (who is unaffected) have the trait.

THINK-PAIR-SHARE Question 2

CONCEPTS

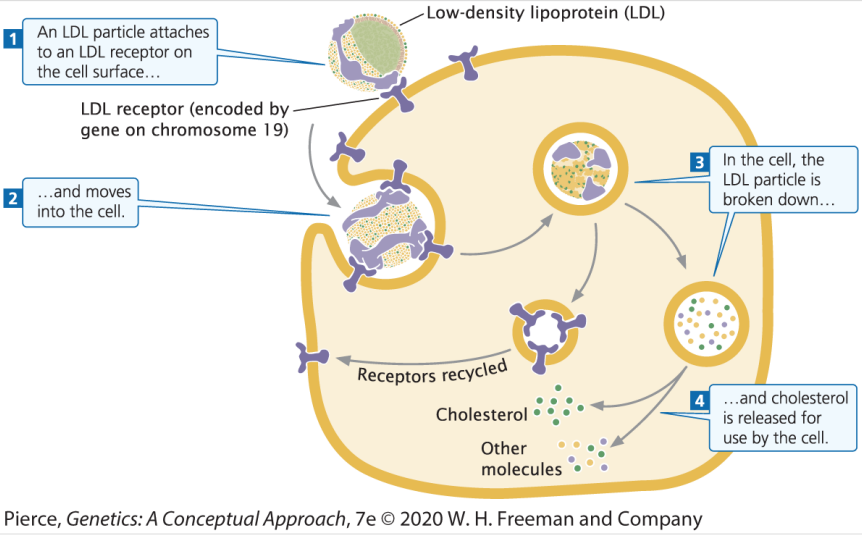
Autosomal dominant traits appear in both sexes with equal frequency. An affected person has an affected parent (unless the person carries new mutations), and the trait does not skip generations. Unaffected people do not transmit the trait.

✔ CONCEPT CHECK 2

When might you see an autosomal dominant trait skip generations?

One condition that is usually considered to be an autosomal dominant trait is familial hypercholesterolemia, an inherited disease in which blood cholesterol is greatly elevated owing to a defect in cholesterol transport. Cholesterol is normally transported throughout the body in small soluble particles called lipoproteins

(Figure 6.6). A principal lipoprotein in the transport of cholesterol is low-density lipoprotein (LDL). When an LDL molecule reaches a cell, it attaches to an LDL receptor, which then moves the LDL through the cell membrane into the cytoplasm, where it is broken down and its cholesterol is released for use by the cell.



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6.6 Low-density lipoprotein (LDL) particles transport cholesterol. The LDL receptor moves LDL from the bloodstream through the cell membrane into the cytoplasm.

Description

The illustration shows a cell with multiple L D L receptors, encoded by gene on chromosome 19, over its surface. A caption reads, “An L D L particle attaches to an L D L receptor on the cell surface and moves into the cell.” The L D L particle then enters into the cell by forming a vesicle. Another caption reads, “In the cell, the L D L particle is broken down and cholesterol is released for use by the cell.” From this vesicle, two vesicles are formed. One vesicle has cholesterol and other molecules and the other has receptors for recycling. The receptors are again recycled and are attached back to the surface. The vesicle with L D L fragments then disintegrates into cholesterol and other molecules.

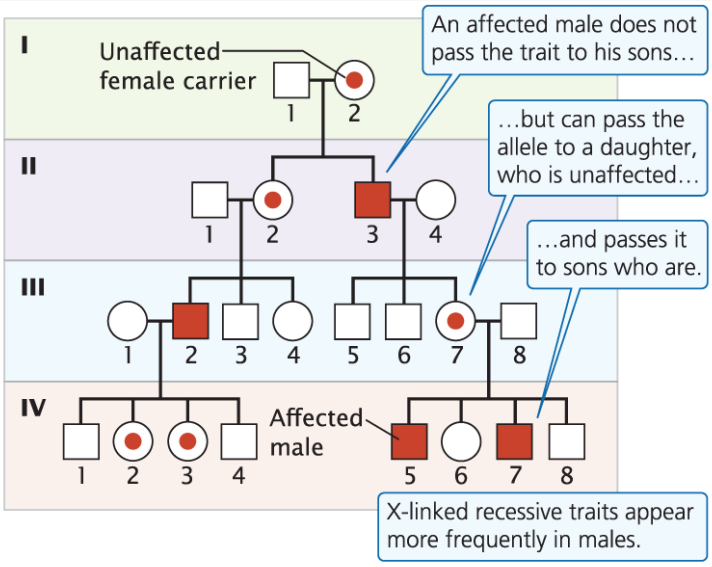
Familial hypercholesterolemia occurs when there is a defect in the gene that normally encodes the LDL receptor. The disease is usually considered an autosomal dominant disorder because heterozygotes are deficient in LDL receptors and have elevated blood levels of cholesterol, which lead to increased risk of coronary artery disease. People who are heterozygous for familial hypercholesterolemia have blood LDL levels that are twice the normal levels and usually have heart attacks by the age of 35.

Very rarely, a person inherits two defective alleles for the LDL receptor. Such individuals don't make any functional LDL receptors; their blood cholesterol levels are more than six times the normal levels, and they may suffer a heart attack as early as age 2 and almost inevitably by age 20. Because homozygotes are more severely affected than heterozygotes, familial hypercholesterolemia is said to be incompletely dominant. However, homozygotes are rarely seen, and the common heterozygous form of the disease appears as a simple dominant trait in most pedigrees.

X-Linked Recessive Traits

X-linked recessive traits have a distinctive pattern of inheritance (Figure 6.7). First, these traits appear more frequently in males than in females because males need inherit only a single copy of the allele to display the trait, whereas females must inherit two copies of the allele, one from each parent, to be affected. Second, because a male

inherits his X chromosome from his mother, affected males are usually born to unaffected mothers who carry an allele for the trait. Because the trait is passed from unaffected female to affected male to unaffected female, it tends to skip generations (see Figure 6.7). When a woman is heterozygous, approximately half of her sons will be affected and half of her daughters will be unaffected carriers. For example, we know that females I-2, II-2, and III-7 in Figure 6.7 are carriers because they transmit the trait to approximately half of their sons. Individuals like these females, whose heterozygous genotype can be definitively determined from the pedigree, are called *obligate carriers*.



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6.7 X-linked recessive traits appear more often in males than in females and are not passed from father to son. Obligate carriers are indicated in this pedigree to help illustrate the X-linked recessive mode of inheritance.

Description

The first generation shows a couple with an unaffected male and a female with a red dot inside the circle labeled as an unaffected female carrier. This couple mates and has 2 offspring: an affected male and a carrier female offspring. A caption reads, “An affected male does not pass the trait to his sons but can pass the allele to a daughter, who is unaffected and passes it to sons who are.” The female carrier in generation 2 mates with an unaffected male and has 1 unaffected daughter and 2 sons, one of which is affected. The affected male from generation 2 mates with an unaffected female and has two unaffected sons and a carrier unaffected daughter. An affected son of the carrier female from generation 2 mates with an unaffected female and has 2 carrier unaffected daughters and 2 unaffected sons in generation 4. A carrier daughter of the affected male from generation 2 mates with an unaffected male and has 2 affected sons, 1 unaffected daughter, and an unaffected son in generation 4. A text below reads, “X-linked recessive traits appear more frequently in males.”

A third important characteristic of X-linked recessive traits is that they are not passed from father to son, because a son inherits his father’s Y chromosome, not his X. In Figure 6.7, there is no case in which both a father and his son are affected. All daughters of an affected man, however, will be carriers (if their mother is homozygous for the normal allele). When a woman displays an X-linked recessive trait, she must be homozygous for the trait, and all her sons will also display the trait.

CONCEPTS

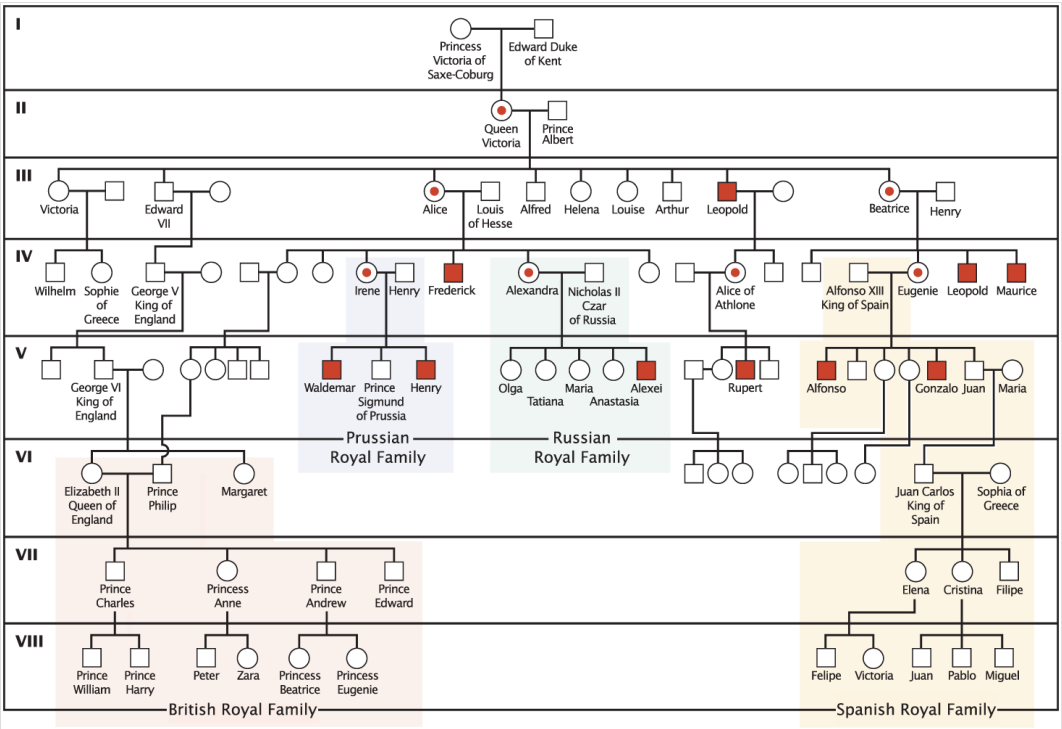
X-linked recessive traits appear more often in males than in females and are not passed from father to son. Affected sons are usually born to unaffected mothers who are carriers of the gene for the trait; thus, X-linked recessive traits tend to skip generations.

✔ CONCEPT CHECK 3

How can you distinguish between an autosomal recessive trait with higher penetrance in males and an X-linked recessive trait?

An example of an X-linked recessive trait in humans is hemophilia A, also called classic hemophilia. Hemophilia results from the absence of a protein necessary for blood to clot. The complex process of blood clotting consists of a cascade of reactions that includes more than 13 different clotting factors. For this reason, there are several types of clotting disorders, each due to a glitch in a different step of the clotting pathway.

Hemophilia A results from abnormal or missing factor VIII, one of the proteins in the clotting cascade. The gene for factor VIII is located on the tip of the long arm of the X chromosome, so hemophilia A is an X-linked recessive disorder. People with hemophilia A bleed excessively; even small cuts and bruises can be life threatening. Spontaneous bleeding occurs in joints such as elbows, knees, and ankles, producing pain, swelling, and erosion of the bone. Fortunately, bleeding in people with hemophilia A can be controlled by administering concentrated doses of factor VIII. The inheritance of hemophilia A is illustrated by the family of Queen Victoria of England (**Figure 6.8**).



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6.8 Classic hemophilia in the royal families of Europe. This blood-clotting disorder is inherited as an X-linked recessive trait.

Description

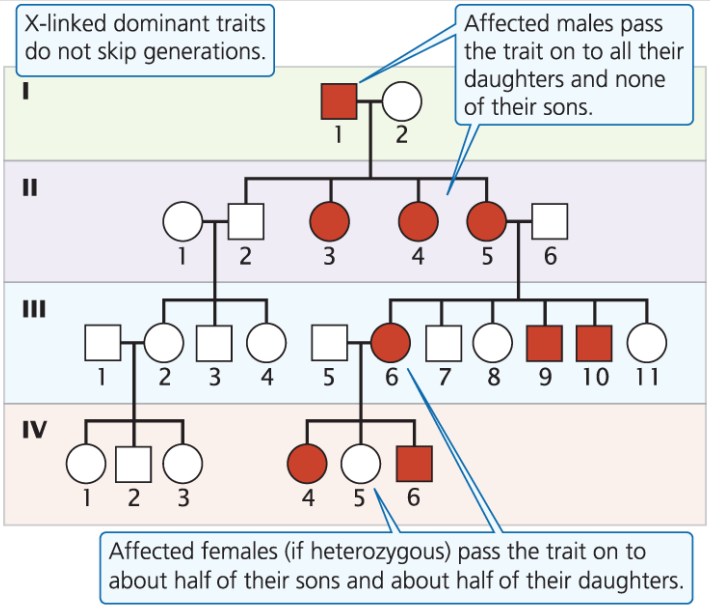
The pedigree includes the names of the royal family members. It starts with Princess Victoria of Saxe-Coburg and Edward Duke of Kent, who are both unaffected. Their daughter, Queen Victoria, becomes a carrier and mates with Prince Albert who is unaffected. They have 9 children in generation 3: Victoria (unaffected), Edward the Seventh (unaffected), Alice (unaffected carrier), Alfred (unaffected), Helena (unaffected), Louise (unaffected), Arthur (unaffected), Leopold (affected), and Beatrice (unaffected carrier). Victoria mates with an unaffected male and has 1 son named Wilhelm and 1 daughter named Sophie of Greece. Edward the Seventh mates with an unaffected female and has 1 son named George the Fifth King of England. Alice mates with Louis of Hessen and has 3 unaffected daughters, 2 carrier but unaffected daughters named Irene and Alexandra, and 1 affected son named Frederick. Leopold mates with an unaffected female and has 1 unaffected but carrier daughter named Alice of Athlone. Beatrice mates with unaffected Henry and has 1 unaffected but carrier daughter named Eugenie, 1 unaffected son, 2 affected sons named Leopold and Maurice. George the Fifth King of England mates with an unaffected female and has two unaffected sons, one of whom is named George the

Sixth King of England. One unaffected daughter of Alice from generation 3 mates with an unaffected male and has 2 unaffected sons and 2 unaffected daughters where one of these daughter has a son named Prince Philip. Irene from generation 4 mates with unaffected Henry and has 2 affected sons named Waldemar and Henry and 1 unaffected son named Prince Sigmund of Prussia. This family is labeled as Prussian Royal Family. Alexandra from generation 4 mates with Nicholas the Second Czar of Russia. They have 4 unaffected daughters named Olga, Tatiana, Marie, Anastasia, and an affected son named Alexis. This is the Russian Royal Family. Alice of Athlone from generation 4 mates with an unaffected male and has 1 unaffected daughter, 1 affected son named Rupert, and 1 unaffected son. Eugenie from generation 4 mates with Alfonso the Thirteenth King of Spain and has 2 affected sons named Alfonso and Gonzalo, 2 unaffected daughters and 2 unaffected sons one of whom is named Juan. George the Sixth King of England mates with an unaffected female and has two unaffected daughters named Elizabeth the Second Queen of England and Margaret. One unaffected son of Alice of Athlone has 2 unaffected daughters and 1 unaffected son in generation 6. One unaffected daughter of Eugenie from generation 4 has 2 unaffected daughters and 1 unaffected son in generation 6. Another unaffected daughter of Eugenie has 1 unaffected daughter in generation 6. Juan mates with unaffected Maria and has an unaffected son named Juan Carlos King of Spain. Elizabeth the Second Queen of England mates with Prince Philip and has 3 unaffected sons named Prince Charles, Prince Andrew, Prince Edward, and 1 unaffected daughter named Princess Anne. Juan Carlos King of Spain from generation 6 mates with unaffected Sophia of Greece and has 2 unaffected daughters named Elena, Cristina, and 1 unaffected son named Filipe. Prince Charles has two unaffected sons named Prince William and Prince Harry in generation 8. Princess Anne has 1 unaffected son named Peter and 1 unaffected daughter named Zara in generation 8. Prince Andrew has two unaffected daughters named Princess Beatrice and Princess Eugenie. This family from Elizabeth the Second Queen of England is British Royal Family. Elena has 1 unaffected daughter named Victoria and 1 unaffected son named Felipe in generation 8. Cristina has 3 unaffected sons named Juan, Pablo, and Miguel in generation 8. This family starting from Alfonso the Eighth King of Spain is Spanish Royal Family. This pedigree chart shows 10 affected males and 7 unaffected but carrier females. Through the few generations shown in the pedigree, only 1 male out of 10 positive for hemophilia mated and had offspring.

X-Linked Dominant Traits

X-linked dominant traits appear in both males and females, although they often appear more frequently in females than in males. Each person with an X-linked dominant trait must have an affected parent (unless the person possesses a new mutation or the trait has incomplete penetrance). X-linked dominant traits do not skip generations (**Figure 6.9**); affected men pass the trait to all their daughters and none of their sons, as is seen in the children of I-1 in Figure 6.9. In contrast, affected women (if heterozygous) pass the trait to about half of their sons and about half of their daughters, as seen in the children of III-6 in the pedigree. As with X-linked recessive traits, a male inherits an X-linked dominant trait only from his mother; the trait is not passed from father to son. This fact is what distinguishes X-linked dominant inheritance from autosomal dominant inheritance, in which a male can inherit the trait from his father. A female, on the other hand, inherits an X chromosome from both her mother and her father, so females can receive an X-linked dominant trait from either parent.

THINK-PAIR-SHARE Question 3



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6.9 X-linked dominant traits affect both males and females. An affected male must have an affected mother.

Description

The pedigree chart shows generation 1 has a couple with affected male and unaffected female. A text reads, “X-linked dominant traits do not skip generations.” A caption reads, “Affected males pass the trait on to all their daughters and none of their sons.” The couple has 3 affected daughters and 2 unaffected sons in generation 2. One unaffected male from generation 2 mates with an unaffected female and has 2 unaffected daughters and 1 unaffected son in generation 3. One of the affected females from generation 2 mates with an unaffected male and has 1 affected daughter, 2 unaffected daughters, 1 unaffected son, and 2 affected sons. A caption reads, “Affected females (if heterozygous) pass the trait on to about half of their sons and about half of their daughters.” One unaffected daughter of the unaffected male from generation 2 mates with an unaffected male and has 1 unaffected son and 2 unaffected daughters in generation 4. The affected daughter of the affected female from generation 2 mates with an unaffected man and has 1 affected daughter, 1 unaffected daughter, and 1 affected son in generation 4.

CONCEPTS

X-linked dominant traits affect both males and females. Affected males must have affected mothers (unless the males possess a new mutation), and they pass the trait to all their daughters.

✔ CONCEPT CHECK 4

A male affected with an X-linked dominant trait will have what proportion of offspring affected with the trait?

a. $^1/_2$ sons and $^1/_2$ daughters
b. All sons and no daughters
c. All daughters and no sons
d. $^3/_4$ daughters and $^1/_4$ sons

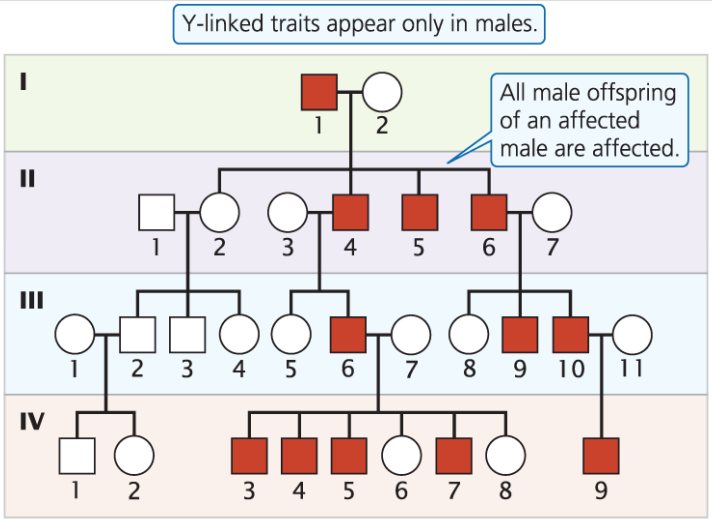
An example of an X-linked dominant trait in humans is hypophosphatemia, or familial vitamin-D-resistant rickets. People with this trait have features that superficially resemble those produced by rickets: bone deformities, stiff spines and joints, bowed legs, and mild growth deficiencies. This disorder, however, is resistant to treatment with vitamin D, which normally cures rickets. X-linked hypophosphatemia results from the defective transport of phosphate, especially in cells of the kidneys. People with this disorder excrete large amounts of phosphate in their urine, resulting in low levels of phosphate in the blood and reduced deposition of minerals in the bone. The disorder is treated with high doses of calcitriol (a hormonally active form of vitamin D) and phosphate. As

is common with X-linked dominant traits, males with hypophosphatemia are often more severely affected than females.

Y-Linked Traits

Y-linked traits exhibit a specific, easily recognized pattern of inheritance. Only males are affected, and the trait is passed from father to son. If a man is affected, all his male offspring should also be affected, as is the case for I-1, II-4, II-6, III-6, and III-10 of the pedigree in **Figure 6.10**. Y-linked traits do not skip generations. As mentioned in Chapter 4, little genetic information is found on the human Y chromosome. Maleness (encoded by the *SRY* gene) is one of the few traits in humans that has been shown to be Y linked. Because each male has a single Y chromosome, there is only one copy of each Y-linked allele; therefore, Y-linked traits are neither dominant nor recessive.

THINK-PAIR-SHARE Question 4



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6.10 Y-linked traits appear only in males and are passed from a father to all his sons.

Description

The pedigree shows generation 1 has a couple where the male is affected and female is unaffected.

A text reads, “Y-linked traits appear only in males.” The couple has 1 unaffected daughter and 3 affected sons in generation 2. A caption reads, “All male offspring of an affected male are affected.” The unaffected female from generation 2 mates with an unaffected male and has 1 unaffected daughter and 2 unaffected sons in generation 3. One of the affected males from generation 2 mates with an unaffected female and has 1 affected son and 1 unaffected daughter in generation 3. Another affected male from generation 2 mates with an unaffected female and has 2 affected sons and 1 unaffected daughter in generation 3. An unaffected male, son of unaffected female from generation 2, mates with an unaffected female and has 1 unaffected son and 1 unaffected daughter in generation 4. One affected son of affected male from generation 2 mates with an unaffected female and has 4 affected sons and 2 unaffected daughters in generation 4. Another affected son of the affected male from generation 2 mates with an unaffected female and has 1 affected son in generation 2.

CONCEPTS

Y-linked traits appear only in males and are passed from a father to all his sons.

✔ CONCEPT CHECK 5

What features of a pedigree would distinguish between a Y-linked trait and a trait that is rare, autosomal dominant, and sex-limited to males?

The major characteristics of autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant, and Y-linked traits are summarized in Table 6.1. ❯ **TRY** PROBLEM 22

TABLE 6.1 Pedigree characteristics of autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant, and Y-linked traits

Autosomal Recessive Trait

1. Usually appears in both sexes with equal frequency.
2. Tends to skip generations.
3. Affected offspring are usually born to unaffected parents.
4. When both parents are heterozygous, approximately one-fourth of the offspring will be affected.
5. Appears more frequently among the children of consanguineous marriages.

Autosomal Dominant Trait

1. Usually appears in both sexes with equal frequency.
2. Both sexes transmit the trait to their offspring.
3. Does not skip generations.
4. Affected offspring must have an affected parent unless they possess a new mutation.
5. When one parent is affected (heterozygous) and the other parent is unaffected, approximately half of the offspring will be affected.
6. Unaffected parents do not transmit the trait.

X-Linked Recessive Trait

1. Usually more males than females are affected.
2. Affected sons are usually born to unaffected mothers; thus, the trait skips generations.
3. Approximately half of a carrier (heterozygous) mother's sons are affected.
4. Never passed from father to son.
5. All daughters of affected fathers are carriers.

X-Linked Dominant Trait

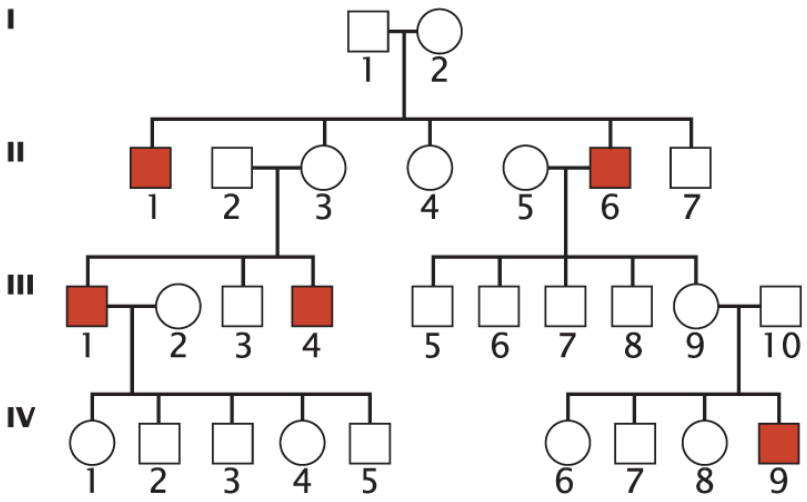
1. Both males and females are usually affected; often, more females than males are affected.
2. Does not skip generations. Affected sons must have an affected mother; affected daughters must have either an affected mother or an affected father.
3. Affected fathers pass the trait to all their daughters.
4. Affected mothers (if heterozygous) pass the trait to half of their sons and half of their daughters.

Y-Linked Trait

1. Only males are affected.
2. Passed from father to all sons.
3. Does not skip generations.

WORKED PROBLEM

The following pedigree represents the inheritance of a rare disorder in an extended family. What is the most likely mode of inheritance for this disease? (Assume that the trait is fully penetrant.)



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Description

All of the affected members are males. 2 in generation 2, 2 in generation 3, and 1 in generation 4. The first generation, both parents are unaffected. They have 5 offspring: 2 positive males, 2 negative females, and 1 negative male. One negative female and 1 positive male mate and have offspring. The female mates a negative male, and they have 2 positive male and 1 negative male offspring. The positive male from generation 2 mates a negative female, and they have 5 negative offspring: 4 male and 1 female. The female mates a negative male, and they have 1 positive male, 2 negative female, and 1 negative male offspring.

Solution Strategy

What information is required in your answer to the problem?

The most likely mode of inheritance for the trait shown in the pedigree.

What information is provided to solve the problem?

- The pedigree, which includes information about the sex and family relationships of affected individuals.
- The trait is rare.

Solution Steps

To answer this question, we should consider each mode of inheritance and determine which, if any, we can eliminate. The trait appears only in males, so autosomal dominant and autosomal recessive modes of inheritance are unlikely because traits with these modes appear equally in males and females. Additionally, autosomal dominance can be eliminated because some affected individuals do not have an affected parent.

The trait is observed only among males in this pedigree, which might suggest Y-linked inheritance. However, affected men should pass a Y-linked trait to all their sons, which is not the case here; II-6 is an affected man who has four unaffected male offspring. Therefore, we can eliminate Y-linked inheritance.

X-linked dominance can be eliminated because affected men should pass an X-linked dominant trait on to all their female offspring, and II-6 has an unaffected daughter (III-9).

X-linked recessive traits often appear more commonly in males, and affected males are usually born to unaffected female carriers; the pedigree shows this pattern of inheritance. If the disorder is an X-

linked trait, about half the sons of a heterozygous carrier mother should be affected. II-3 and III-9 are suspected carriers, and about half of their male children (three of five) are affected. Another important characteristic of an X-linked recessive trait is that it is not passed from father to son. We observe no father-to-son transmission in this pedigree. X-linked recessive is therefore the most likely mode of inheritance.

» For additional practice, try to determine the mode of inheritance for the pedigrees in **Problem 25** at the end of the chapter.

Genetic Mosaicism

A factor that can complicate the interpretation of pedigrees is the presence of mosaicism. A long-held assumption in genetics was that every cell in the body contains the same genetic information. Yet from time to time, geneticists discovered exceptions to this rule. In 1976, researchers published the curious case of a woman who had two different blood types. Most of her red blood cells were type O, but a few cells were type A. In 1998, a 52-year-old woman needed a kidney transplant. When her three children were genetically tested to see if any of them might be a potential donor, two of the children had genotypes that could not have come from their mother, even though she had given birth to them. Further testing revealed that the mother actually had two different types of cells in her body: the

genotype of her blood cells was different from that of her skin and some organs. These individuals, in which different cells of the body have different genetic constitutions, are referred to as **genetic mosaics** or chimeras.

Genetic mosaicism can arise from a number of different processes. Often, somatic mutations or errors in the separation of chromosomes during early development lead to genetically distinct groups of cells within the body. There can also be exchanges of cells and DNA between twins or between mother and child during pregnancy. Mosaicism was once thought to be rare, but the increasing use of DNA sequencing has revealed that it may be common. For example, in 2012, researchers sequenced skin cells and discovered that 30% of the cells contained genetic variants called copy-number variations (CNVs, see Chapter 8). Another study examined CNVs in diverse tissues from six people and also found major differences in CNVs, even within a single person.

6.3 Studying Twins and Adoptions Can Help Assess the Importance of Genes and Environment

Twins and adoptions provide natural experiments for separating the effects of genes and environmental factors on differences in traits. These two techniques have been widely used in genetic studies.

Types of Twins

Twins are of two types. **Dizygotic (nonidentical) twins** arise when two separate eggs are fertilized by two different sperm, producing genetically distinct zygotes. **Monozygotic (identical) twins** result when a single egg, fertilized by a single sperm, splits early in development into two separate embryos.

Because monozygotic twins result from a single egg and sperm (a single, "mono," zygote), they're genetically identical (except for epigenetic differences—see Chapter 21—or rare somatic mutations); thus, identical twins share 100% of their genes (**Figure 6.11a**). Dizygotic twins (**Figure 6.11b**), on the other hand, share, on average, only 50% of their genes, which is the same percentage that any pair of siblings shares. Like other siblings, dizygotic twins may be of the same sex or of different sexes. The only difference between dizygotic

twins and other siblings is that dizygotic twins are the same age and shared the same uterine environment. Dizygotic twinning often runs in families, and the tendency to produce dizygotic twins is influenced by both heredity and environmental factors. There appears to be little genetic tendency for producing monozygotic twins. The frequency of dizygotic twinning in the United States has doubled since 1980, largely because of fertility treatments, which often stimulate women to ovulate multiple eggs.

THINK-PAIR-SHARE Question 5

(a)

(b)

Part a: f4foto/Alamy. Part b: Courtesy of Randi Rossignol.

6.11 Two types of twins. Monozygotic twins (a) are identical; dizygotic twins (b) are nonidentical.

CONCEPTS

Dizygotic twins develop from two eggs fertilized by two separate sperm; on average, they share 50% of their genes. Monozygotic twins develop from a single egg, fertilized by a single sperm, that splits into two embryos; they share 100% of their genes.

✔ CONCEPT CHECK 6

Why are monozygotic twins genetically identical, whereas dizygotic twins share only 50% of their genes on average?

a. Monozygotic twins tend to look more similar.
b. Monozygotic twins develop from two different eggs fertilized by the same sperm, whereas dizygotic twins develop from two eggs fertilized by two different sperm.
c. Monozygotic twins develop from a single egg fertilized by one sperm, whereas dizygotic twins develop from a single egg fertilized by two different sperm.
d. Monozygotic twins develop from a single egg fertilized by a single sperm, whereas dizygotic twins develop from two eggs fertilized by two different sperm.

Concordance in Twins

Comparisons of dizygotic and monozygotic twin pairs can be used to assess the importance of genetic and environmental factors in producing differences in a characteristic. Such assessments are often made by calculating the concordance for a trait. If both members of a twin pair have a trait, the twins are said to be *concordant*; if only one member of the pair has the trait, the twins are said to be *discordant*. **Concordance** is the percentage of twin pairs that are concordant for a trait. Because identical twins share 100% of their genes and dizygotic twins share, on average, only 50%, genetically influenced traits should exhibit higher concordance in monozygotic twins. For instance, when one member of a monozygotic twin pair has epilepsy (**Table 6.2**), the other twin of the pair has epilepsy about 59% of the time; thus, the monozygotic concordance for epilepsy is 59%. When a dizygotic twin has epilepsy, however, the other twin has epilepsy only 19% of the time (19%

dizygotic concordance). The higher concordance in monozygotic twins suggests that genes influence epilepsy, a finding supported by the results of other family studies of this disease. In contrast, the concordance of death from acute infection is similar in monozygotic and dizygotic twin pairs, suggesting that variation in death from infection has little inherited tendency. Concordances for several additional human traits and diseases are listed in Table 6.2.

TABLE 6.2 Concordance of monozygotic and dizygotic twins for several traits

	Concordance (%)	
Trait	**Monozygotic**	**Dizygotic**
Heart attack (males)	39	26
Heart attack (females)	44	14
Bronchial asthma	47	24
Cancer (all sites)	12	15
Epilepsy	59	19
Death from acute infection	7.9	8.8
Rheumatoid arthritis	32	6
Multiple sclerosis	28	5

[Data from: (1 and 2) B. Havald and M. Hauge, U.S. Public Health Service Publication 1103 (1963), pp. 61–67; (3, 4, 5, and 6) B. Havald and M. Hauge, *Genetics and the Epidemiology of Chronic Diseases* (U.S. Department of Health, Education, and Welfare, 1965); (7) J. S. Lawrence, *Annals of Rheumatic Diseases* 26:357–379, 1970; (8) G. C. Ebers et al., *American Journal of Human Genetics* 36:495, 1984.]

The use of twins in genetic research rests on the important assumption that when concordance for monozygotic twins is greater

than that for dizygotic twins, it is because monozygotic twins are more similar in their genes and not because they have experienced a more similar environment. The degree of environmental similarity between monozygotic twins and dizygotic twins is assumed to be the same. This assumption may not always be correct, particularly for human behaviors. Because they look alike, identical twins may be treated more similarly by parents, teachers, and peers than are nonidentical twins. Evidence of this similar treatment is seen in the past tendency of parents to dress identical twins alike. In spite of this potential complication, twin studies have played a pivotal role in the study of human genetics. › **TRY** PROBLEM 32

THINK-PAIR-SHARE Question 6

A Twin Study of Asthma

To illustrate the use of twins in genetic research, let's consider a study of asthma. Asthma is characterized by constriction of the airways and the secretion of mucus into the air passages, causing coughing, labored breathing, and wheezing (Figure 6.12). Severe cases can be life threatening. Asthma is a major health problem in industrialized countries and appears to be on the rise. The incidence of childhood asthma varies widely around the globe; some of the highest rates (from 21% to 27%) are found in Australia, the United Kingdom, Sweden, and Brazil.

Stockbyte/Getty Images.

6.12 Twin studies show that asthma, characterized by constriction of the airways, is caused by a combination of genetic and environmental factors. Inhalers are often used to deliver asthma medication to the lungs.

A number of environmental stimuli are known to precipitate asthma attacks, including dust, pollen, air pollution, respiratory infections, exercise, cold air, and emotional stress. Allergies frequently accompany asthma, suggesting that asthma is a disorder of the immune system, but the precise relation between immune function and asthma is poorly understood. Numerous studies have shown that genetic factors are important in asthma.

A genetic study of childhood asthma was conducted as part of the Twins Early Development Study in England, an ongoing research project that studies more than 15,000 twin pairs born in the United Kingdom between 1994 and 1996. These twins were assessed for

language, cognitive development, behavioral problems, academic achievement, and other traits at various ages, and the genetic and environmental contributions to a number of their traits were examined. In the asthma study, researchers looked at a sample of 4910 twin pairs at age 4. Parents of the twins were asked whether either of their twins had been prescribed medication to control asthma; those children receiving asthma medication were considered to have asthma. The concordance for the monozygotic twins (65% among 1658 twin pairs) was significantly higher than that for the dizygotic twins (37% among 3252 twin pairs), and the researchers concluded that among the 4-year-olds included in the study, asthma was strongly influenced by genetic factors. The fact that even monozygotic twins were discordant 35% of the time indicates that environmental factors also play a role in asthma.

CONCEPTS

Higher concordance for monozygotic twins than for dizygotic twins indicates that genetic factors play a role in determining differences in a trait. Less than 100% concordance for monozygotic twins indicates that environmental factors play a significant role.

✔ CONCEPT CHECK 7

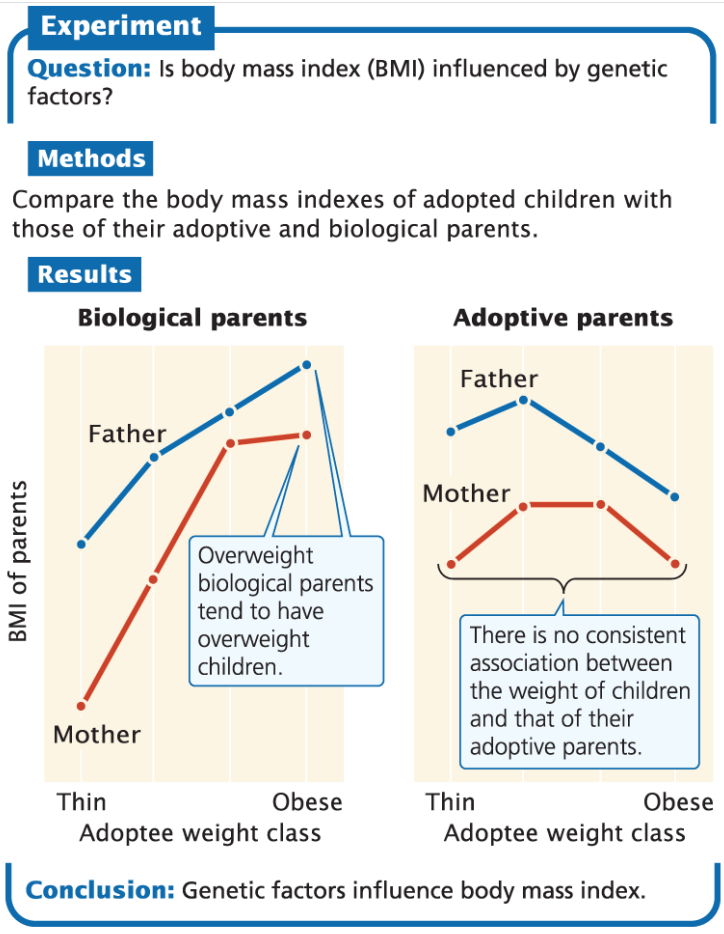
A trait exhibits 100% concordance for both monozygotic and dizygotic twins. What conclusion can you draw about the role of genetic factors in determining differences in the trait?

a. Genetic factors are extremely important.
b. Genetic factors are somewhat important.
c. Genetic factors are unimportant.
d. Both genetic and environmental factors are important.

Adoption Studies

Another technique used by geneticists to analyze human inheritance is the study of families involved in adoptions. This approach is one of the most powerful for distinguishing the effects of genes and environment on characteristics.

For a variety of reasons, many children are separated from their biological parents soon after birth and adopted by adults with whom they have no genetic relationship. These adoptees share no more genes with their adoptive parents, on average, than any two randomly chosen individuals share; however, they do share an environment with their adoptive parents. In contrast, the adoptees share 50% of their genes with each of their biological parents, but do not share the same environment with them. If adoptees and their adoptive parents show similarities in a characteristic, these similarities can be attributed to environmental factors. If, on the other hand, adoptees and their biological parents show similarities, these similarities are probably due to genetic factors. Comparisons of adoptees with their adoptive parents and with their biological parents can therefore help define the roles of genetic and environmental factors in the determination of human variation. For example, adoption studies were instrumental in showing that schizophrenia has a genetic basis. Adoption studies have also shown that variation in obesity is at least partly influenced by genetics (**Figure 6.13**).



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6.13 Adoption studies demonstrate that variation in obesity is influenced by genetics. In these studies, obesity was measured by body mass index (BMI). [Data from the *New England Journal of Medicine* 314:195, 1986.]

Description

The illustration shows the following question for the experiment: Is body mass index (B M I) in uenced by genetic

factors? The methods below include the text, Compare the body mass indexes of adopted children with

those of their adoptive and biological parents. The results include two line graphs: The first graph shows results for biological parents. The horizontal axis represents Adoptee weight class and has two markings from left to right: thin and obese. The vertical axis represents

B M I of parents. The graph shows the following information: The line representing mother rises upwards steeply from a little above the thin marking and then its slope becomes almost plateauing as it approaches obese. The line representing father is above the line for mothers. It rises steeply upwards, then becomes a more shallow but consistent incline. A caption referring to both lines reads, “Overweight biological parents tend to have overweight children.”

The second graph shows results for adoptive parents. The horizontal and vertical axis represents the same parameters. The graph shows the following information: The line representing mother rises upwards from high above the thin marking, plateaus between the markings, then declines to reach the same level as the start point above the obese marking. The line representing father is higher than the start point for mothers, rises a little upwards, then enters a steady decline as it approaches obese, but remains above the line representing mother. A caption referring to both lines reads, “There is no consistent association between the weight of children and that of their adoptive parents.” The conclusion states that genetic factors in uence body mass index.

Adoption studies assume that the environments of biological and adoptive families are independent (i.e., not more alike than would be expected by chance). This assumption may not always be correct because adoption agencies carefully choose adoptive parents and may select a family that resembles the adoptee’s biological family. Thus, some of the similarity between adoptees and their biological parents may be due to these similar environments and not to shared genetic factors. In addition, adoptees and their biological mothers share the same environment during prenatal development. **› TRY PROBLEM 35**

CONCEPTS

Similarities between adoptees and their genetically unrelated adoptive parents indicate that environmental factors affect variation in a particular characteristic; similarities between adoptees and their biological parents indicate that genetic factors influence variation in the characteristic.

✔ CONCEPT CHECK 8

What assumptions underlie the use of adoption studies in genetics?

a. Adoptees have no contact with their biological parents after birth.
b. The adoptive parents and biological parents are not related.
c. The environments of biological and adoptive parents are independent.
d. All of the above

6.4 Genetic Counseling and Genetic Testing Provide Information to Those Concerned about Genetic Diseases and Traits

Our knowledge of human genetic diseases and disorders has expanded rapidly in recent years. The Online Mendelian Inheritance in Man Web site now lists more than 24,000 human genetic diseases, disorders, genes, and traits that have a simple genetic basis. Research has provided a great deal of information about the inheritance, chromosomal location, biochemical basis, and symptoms of many of these genetic traits, diseases, and disorders. This information is often useful to people who have a genetic condition.

Genetic Counseling

Genetic counseling is a field that provides information to patients with genetic disorders and others who are concerned about hereditary conditions. It is an educational process that helps patients and family members deal with many aspects of having a genetic condition: its diagnosis, symptoms and treatment, and mode of inheritance. Genetic counseling also helps patients and their families cope with the psychological and physical stress that may be

associated with the disorder. Clearly, all of these considerations cannot be handled by a single health professional; most genetic counseling is done by a team that may include genetic counselors, physicians, medical geneticists, and laboratory personnel. Table 6.3 lists some common reasons for seeking genetic counseling.

TABLE 6.3 Common reasons for seeking genetic counseling

1. A person knows of a genetic disease in the family.
2. A couple has given birth to a child with a genetic disease, birth defect, or chromosome abnormality.
3. A couple has a child who is intellectually disabled or has a close relative who is intellectually disabled.
4. An older woman becomes pregnant or wants to become pregnant. There is disagreement about the age at which a prospective mother who has no other risk factors should seek genetic counseling; many experts suggest that it should be age 35 or older.
5. Husband and wife are closely related (e.g., first cousins).
6. A couple experiences difficulties achieving a successful pregnancy.
7. A pregnant woman is concerned about exposure to an environmental substance (drug, chemical, or virus) that causes birth defects.
8. A couple needs assistance in interpreting the results of a prenatal or other test.
9. Both prospective parents are known carriers for a recessive genetic disease or both belong to an ethnic group with a high frequency of a genetic disease.

Genetic counseling usually begins with a diagnosis of a condition that may have a genetic basis. On the bases of a physical examination, biochemical tests, DNA testing, chromosome analysis, family history, and other information, a physician determines the cause of the condition. An accurate diagnosis is critical because appropriate treatment and the probability of passing the condition

on depend on the diagnosis. For example, there are a number of different types of dwarfism, caused by chromosome abnormalities, single-gene mutations, hormonal imbalances, and environmental factors. People who have dwarfism resulting from an autosomal dominant gene have a 50% chance of passing the condition on to their children, whereas people who have dwarfism caused by a rare recessive gene have a low likelihood of doing so.

When the nature of the condition is known, a genetic counselor meets with the patient and members of the patient's family and explains the diagnosis. A family pedigree may be constructed, and the probability of transmitting the condition to future generations can be calculated for different family members. The counselor helps the family interpret the genetic risks and explains various available reproductive options, including prenatal diagnosis, artificial insemination, and in vitro fertilization. Family members often have questions about genetic testing that may be available to help determine whether they carry a particular genetic mutation. The counselor helps them decide whether genetic testing is appropriate for them and which tests to apply. After the test results are in, the genetic counselor usually helps family members interpret the results.

A family's decision about future pregnancies frequently depends on the magnitude of the genetic risk, the severity and effects of the condition, the importance they place on having children, and their religious and cultural views. Traditionally, genetic counselors have

been trained to apply *nondirected* counseling, which means that they provide information and facilitate discussion but do not bring their own opinions and values into the discussion. The goal of nondirected counseling is for the family to reach its own decision on the basis of the best available information.

Because of the growing number of genetic tests available and the complexity of assessing genetic risk, there is now some movement away from completely nondirected counseling. The goal is still to provide the family with information about all options and to reach the decision that is best for them, but that goal may sometimes require the counselor to recommend certain options, much as a physician recommends the most appropriate medical treatments for his or her patient.

Who does genetic counseling? In the United States, over 7000 health professionals are certified in genetics by the American Board of Medical Genetics and Genomics or the American Board of Genetic Counseling. About half of them are specifically trained in genetic counseling, usually through a special two-year master's degree program that provides education in both genetics and counseling. Most of the remainder are physicians and scientists certified in medical or clinical genetics. Because of the shortage of genetic counselors and medical geneticists, information about genetic testing and genetic risk is often conveyed by primary care physicians, nurses, and social workers. **› TRY PROBLEM 10**

CONCEPTS

Genetic counseling is an educational process that provides patients and their families with information about a genetic condition, its medical implications, the mode of inheritance, and reproductive options.

Genetic Testing

The ultimate goal of genetic testing is to recognize the potential for a genetic condition at an early stage. In some cases, genetic testing allows people to make informed choices about reproduction. In other cases, genetic testing allows early intervention that may lessen or even prevent the development of a condition. For those who know that they are at risk for a genetic condition, genetic testing may help alleviate anxiety associated with the uncertainty of their situation. Genetic testing includes prenatal testing and postnatal testing.

Prenatal genetic tests are those that are conducted before birth and now include procedures for diagnosing several hundred genetic diseases and disorders (**Table 6.4**). The major purpose of prenatal tests is to provide families with the information that they need to make choices during pregnancies and, in some cases, to prepare for the birth of a child with a genetic condition. Several approaches to prenatal diagnosis are described in the following sections.

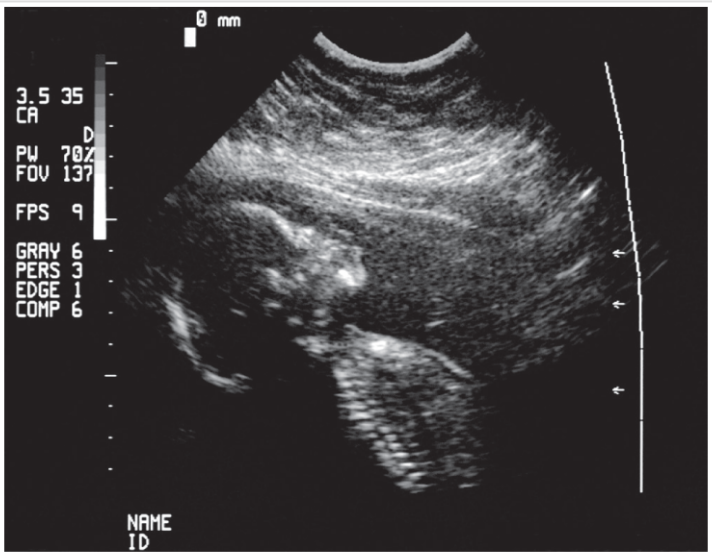
TABLE 6.4 Examples of genetic diseases and disorders that can be detected prenatally and the techniques used in their detection

Disorder	Method of Detection
Chromosome abnormalities	Examination of a karyotype from cells obtained by amniocentesis or chorionic villus sampling. Some forms can be detected by DNA analysis of maternal blood.
Cleft lip and palate	Ultrasound
Cystic fibrosis	DNA analysis of cells obtained by amniocentesis or chorionic villus sampling
Dwarfism	Ultrasound or X-ray; some forms can be detected by DNA analysis of cells obtained by amniocentesis or chorionic villus sampling
Hemophilia	Fetal blood sampling* or DNA analysis of cells obtained by amniocentesis or chorionic villus sampling
Lesch–Nyhan syndrome	Biochemical tests on cells obtained by amniocentesis or chorionic villus sampling
Neural-tube defects	Initial screening with maternal blood test, followed by biochemical tests on amniotic fluid obtained by amniocentesis or by the detection of birth defects with the use of ultrasound
Osteogenesis imperfecta	Ultrasound or X-ray
Phenylketonuria	DNA analysis of cells obtained by amniocentesis or chorionic villus sampling
Sickle-cell anemia	Fetal blood sampling* or DNA analysis of cells obtained by amniocentesis or chorionic villus sampling
Tay–Sachs disease	Biochemical tests on cells obtained by amniocentesis or chorionic villus sampling

*A sample of fetal blood is obtained by inserting a needle into the umbilical cord.

ULTRASONOGRAPHY

Some genetic conditions can be detected through direct visualization of the fetus. This is most commonly done by **ultrasonography**—usually referred to as *ultrasound*. In this technique, high-frequency sound is beamed into the uterus; when the sound waves encounter dense tissue, they bounce back and are transformed into a picture (**Figure 6.14**). In this way, the size and position of the fetus can be determined, and conditions such as neural-tube defects (defects in the development of the spinal column and the skull) and skeletal abnormalities can be detected. Ultrasound is a standard procedure performed during pregnancy to estimate the age of the fetus, determine its sex, and check for the presence of developmental disorders or other problems.

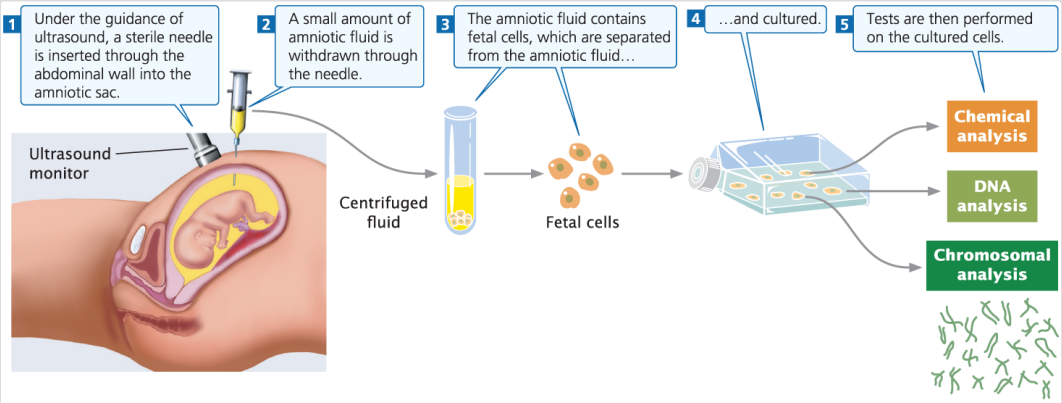


PhotoDisc/Media Bakery.

6.14 Ultrasonography can be used to detect some genetic disorders in a fetus and to locate the fetus during amniocentesis and chorionic villus sampling.

AMNIOCENTESIS

Traditional prenatal testing requires fetal tissue, which can be obtained in several ways. The most widely used method is **amniocentesis**, a procedure for obtaining a sample of amniotic fluid from the uterus of a pregnant woman (Figure 6.15). Amniotic fluid—the substance that fills the amniotic sac and surrounds the developing fetus—contains fetal cells that can be cultured and used for genetic testing.



6.15 Amniocentesis is a procedure for obtaining fetal cells for genetic testing.

Description

The illustration shows a woman's stomach with a fetus inside on the left. An ultrasound monitor is placed on top of the stomach and a needle is inserted into the amniotic sac. The first caption pointing towards the ultrasound monitor reads, "Under the guidance of ultrasound, a sterile needle is inserted through the abdominal wall into the amniotic sac." The second caption pointing towards the needle reads, "A small amount of amniotic fluid is withdrawn through the needle." Centrifuged fluid obtained from the needle is then transferred to a test tube on the right. The third caption reads, "The amniotic fluid contains fetal cells, which are separated from the amniotic fluid and cultured." Round-shaped fetal cells are obtained from amniotic fluid and cultured. The culture is eventually sent for chemical analysis, D N A analysis, and chromosomal analysis. The final caption reads, "Tests are then performed on the cultured cells."

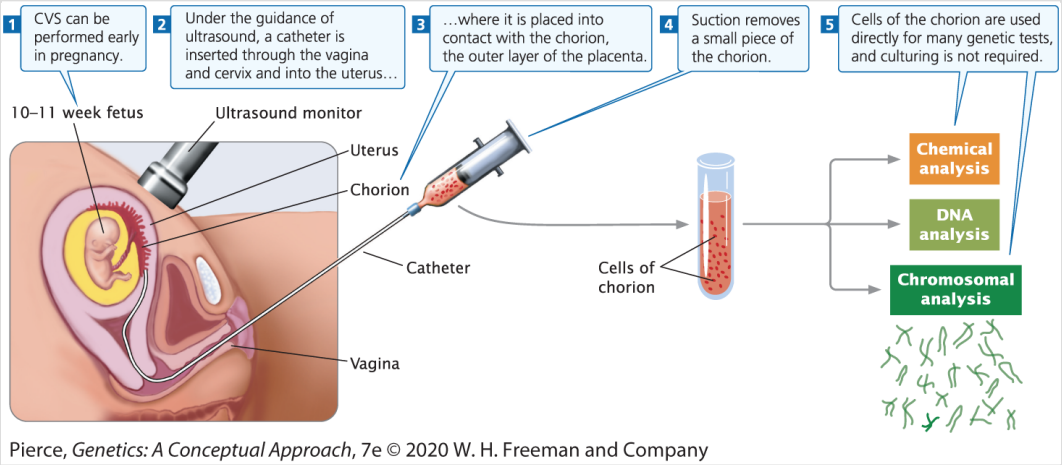
Amniocentesis is routinely performed as an outpatient procedure either with or without the use of a local anesthetic (see Figure 6.15). Genetic tests are then performed on the cultured cells. Complications with amniocentesis (mostly miscarriage) are uncommon, arising in only about 1 in 1600 procedures.

CHORIONIC VILLUS SAMPLING

A major disadvantage of amniocentesis is that it generally cannot be performed until about the 15th to 18th week of a pregnancy (although some obstetricians successfully perform amniocentesis earlier). The cells obtained by amniocentesis must then be cultured before genetic tests can be performed, which requires yet more time. For these reasons, genetic information about the fetus may not be available until the 17th or 18th week of pregnancy. By this stage, if abortion is chosen, it carries a greater risk of complications and is often stressful for the parents. **Chorionic villus sampling (CVS)** can be performed earlier (between the 10th and 12th weeks of pregnancy) and collects a larger amount of fetal tissue, which eliminates the necessity of culturing the cells.

In CVS, a catheter—a soft plastic tube—is placed in contact with the chorion, the outer layer of the placenta (**Figure 6.16**). Suction is then applied, and a small piece of the chorion is removed. Although the chorion is composed of fetal cells, it is a part of the placenta, which is expelled from the uterus after birth; the tissue that is removed is not actually from the fetus. This tissue contains millions of actively

dividing cells that can be used directly in many genetic tests. Chorionic villus sampling has a somewhat higher risk of complications than amniocentesis; the results of several studies suggest that this procedure may increase the incidence of limb defects in the fetus when performed earlier than the 10th week of pregnancy.



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6.16 Chorionic villus sampling (CVS) is another procedure for obtaining fetal cells for genetic testing.

Description

The illustration shows a woman's uterus with a fetus inside on the left. The fetus is labeled as 10 to 11 weeks old. The illustration also shows vagina and chorion marked. The ultra sound monitor is placed on top of the stomach. A thin tube known as catheter is attached to a syringe and is inserted into the vagina and threads through the inner cavity to the right side of the fetus. The first caption pointing towards 10 to 11 week fetus reads, "C V S can be performed early in pregnancy." The second caption reads, "Under the guidance of ultrasound, a catheter is inserted through the vagina and cervix and into the uterus where it is placed into contact with the chorion, the outer layer of the placenta." The next caption pointing towards the syringe reads, "Suction removes a small piece of the chorion." Cells of chorion are obtained from the syringe and are placed in a test tube on the right. These cells are eventually sent for chemical analysis, D N A analysis, and chromosomal analysis.

> The final caption reads, “Cells of the chorion are used directly for many genetic tests, and culturing is not required.”

Fetal cells obtained by amniocentesis or by CVS can be used to prepare a **karyotype**, which is a picture of a complete set of metaphase chromosomes. Karyotypes can be studied for chromosome abnormalities (see Chapter 8). Biochemical analyses can be conducted on fetal cells to determine the presence of particular metabolic products of genes. To detect genetic diseases for which the DNA sequence of the causative gene has been determined, the DNA sequence (DNA testing; see Chapter 19) can be examined for defective alleles.

MATERNAL BLOOD SCREENING TESTS

Increased risk of some genetic conditions can be detected by examining levels of certain substances in the blood of the mother (referred to as a **maternal blood screening tests**). However, these tests do not determine the presence of a genetic problem; rather, they simply indicate that the fetus is at increased risk of a problem and hence are referred to as *screening* tests. When increased risk is detected, follow-up tests (additional blood screening tests, ultrasound, amniocentesis, or all three) are usually conducted.

One substance examined in maternal blood screening tests is α-fetoprotein, a protein that is normally produced by the fetus during

development and is present in fetal blood, amniotic fluid, and the mother's blood during pregnancy. The level of α-fetoprotein is significantly higher than normal when the fetus has a neural-tube defect or one of several other disorders. Some chromosome abnormalities produce lower-than-normal levels of α-fetoprotein. Measuring the amount of α-fetoprotein in the mother's blood gives an indication of these conditions.

One typical test, carried out between the 9th and 13th weeks of pregnancy, measures human chorionic gonadotropin (hCG, a pregnancy hormone) and a substance called pregnancy-associated plasma protein A (PAPP-A). When the fetus has certain chromosome abnormalities, the level of PAPP-A tends to be low and the level of hCG tends to be high. The risk of a chromosome abnormality is calculated on the basis of the levels of hCG and PAPP-A in the mother's blood along with the results of ultrasound. Another test, referred to as the quad screen, is usually conducted in the second trimester of pregnancy and measures the levels of four substances: α-fetoprotein, hCG, estriol, and inhibin. The risk of chromosome abnormalities and certain other birth defects is calculated on the basis of the combined levels of the four substances plus the mother's age, weight, ethnic background, and number of fetuses. The quad screen successfully detects Down syndrome (due to three copies of chromosome 21) approximately 80% of the time and has a false positive rate of about 5%.

NONINVASIVE PRENATAL SCREENING

Prenatal tests that use only maternal blood are highly desirable because they are noninvasive and pose no risk to the fetus. In addition to maternal blood screening tests, which measure chemical substances produced by the fetus or placenta, these noninvasive tests include procedures collectively called **noninvasive prenatal screening (NIPS)**, which directly examines fetal DNA found in maternal blood. These tests can be performed as early as the 9th to 10th week of pregnancy.

During pregnancy, a few fetal cells are released into the mother's circulatory system, where they mix with and circulate with her blood. When these fetal cells break down, they release free-floating fragments of fetal DNA, which can be sequenced and analyzed. By comparing the number of copies of genetic variants found on different chromosomes, it is possible to determine if there are extra copies of some fetal chromosomes present and therefore to screen for Down syndrome, trisomy 18, trisomy 13, and other chromosome abnormalities (see Chapter 8) in the fetus. When a chromosome abnormality is detected, diagnosis is confirmed with amniocentesis or chorionic villus sampling. Research suggests that noninvasive prenatal screening for Down syndrome is more accurate and has a much lower false positive rate than traditional maternal blood screening procedures that rely on levels of substances such as α-fetoprotein, hCG, estriol, and inhibin. Recently, researchers used measurement of cell-free fetal RNA (see RNA sequencing in Chapter

20) to accurately estimate gestational age and estimate the risk of preterm birth.

Prenatal genetic screening tests based on DNA in the mother's blood occasionally reveal information about the mother's genetic makeup or health. For example, sometimes DNA analysis detects the presence of a Y chromosome, but ultrasound reveals a female fetus. In a few rare instances, this finding is due to a previously undiagnosed sex chromosome anomaly of the mother (although there are other potential explanations as well). Occasionally, testing can reveal the presence of undiagnosed cancer in the mother. Whether and how this type of incidental genetic information should be conveyed to the mother, who did not request genetic testing on herself, remains the subject of discussion among genetic counselors.

Noninvasive prenatal screening technology creates the potential in the future to use a single blood sample from the mother to test for hundreds of genetic diseases and even for ordinary traits in the fetus. At the present time, this is technically difficult and beyond routine clinical practice due to the confounding effects of maternal DNA, but researchers have demonstrated that they can sequence the entire fetal genome from a maternal blood sample. This possibility raises a number of social and ethical questions about the use of such information in reproductive decisions.

PREIMPLANTATION GENETIC DIAGNOSIS

Prenatal genetic tests provide today's prospective parents with increasing amounts of information about the health of their future children. New reproductive technologies provide couples with options for using this information. One of these technologies is in vitro fertilization. In this procedure, hormones are used to induce ovulation. The ovulated eggs are surgically removed from the surface of the ovary, placed in a laboratory dish, and fertilized with sperm. The resulting embryo is then implanted in the uterus. Thousands of babies resulting from in vitro fertilization have now been born.

Genetic testing can be combined with in vitro fertilization to allow the implantation of embryos that are free of a specific genetic defect. Called **preimplantation genetic diagnosis (PGD)**, this technique enables people who carry a genetic defect to avoid producing a child with the disorder. For example, if a woman is a carrier of an X-linked recessive disease, approximately half of her sons are expected to have the disease. Through in vitro fertilization and PGD, an embryo without the disorder can be selected for implantation in her uterus.

The procedure begins with the production of several single-celled embryos through in vitro fertilization. The embryos are allowed to divide several times until they reach the 8- or 16-cell stage. At this point, one cell is removed from each embryo and tested for the genetic abnormality. Removing a single cell at this early stage does not harm the embryo. Once the embryos that are free of the disorder

have been identified, a healthy embryo is selected and implanted in the woman's uterus.

Preimplantation genetic diagnosis requires the ability to conduct genetic testing on a single cell. Such testing is possible with the use of the polymerase chain reaction, through which minute quantities of DNA can be amplified (replicated) quickly (see Chapter 19). Once the cell's DNA has been amplified, the DNA sequence is examined. Preimplantation genetic diagnosis has resulted in the birth of thousands of healthy children. Its use raises a number of ethical concerns because it can be used as a means of selecting for or against genetic traits that have nothing to do with medical concerns. For example, it can potentially be used to select for a child with genes for a certain eye color or genes for increased height.

NEWBORN SCREENING

Testing for genetic disorders in newborn infants is called **newborn screening**. All states in the United States and many other countries require by law that newborn infants be tested for some genetic diseases and conditions. The Secretary of the U.S. Department of Health and Human Services recommends mandatory screening for 35 genetic conditions (**Table 6.5**), and many states use this list for newborn testing. These genetic conditions were chosen because early identification can lead to effective treatment. For example, as mentioned in Chapter 5, phenylketonuria is an autosomal recessive disease that, if not treated at an early age, can result in intellectual

disability. But early intervention, through the administration of a modified diet, prevents this outcome.

TABLE 6.5 Genetic conditions recommended for screening in newborns by the Secretary of the U.S. Department of Health and Human Services (The Recommended Uniform Screening Panel Core Conditions, as of July 2018)

Genetic Condition
Propionic acidemia
Methylmalonic acidemia (methylmalonyl-CoA mutase)
Methylmalonic acidemia (cobalamin disorders)
Isovaleric acidemia
3-Methylcrotonyl-CoA carboxylase deficiency
3-Hydroxy-3-methyglutaric aciduria
Holocarboxylase synthase deficiency
β-Ketothiolase deficiency
Glutaric acidemia type I
Carnitine uptake defect/carnitine transport defect
Medium-chain acyl-CoA dehydrogenase deficiency
Very long-chain acyl-CoA dehydrogenase deficiency
Long-chain L-3 hydroxyacyl-CoA dehydrogenase deficiency
Trifunctional protein deficiency
Argininosuccinic aciduria
Citrullinemia, type I
Maple syrup urine disease
Homocystinuria
Classic phenylketonuria
Tyrosinemia, type I

Primary congenital hypothyroidism
Congenital adrenal hyperplasia
S,S disease (sickle cell anemia)
S, β-thalassemia
S,C disease
Biotinidase deficiency
Critical congenital heart disease
Cystic fibrosis
Classic galactosemia
Glycogen storage disease type II (Pompe)
Hearing loss
Severe combined immunodeficiencies
Mucopolysaccharidosis type 1
X-linked adrenoleukodystrophy
Spinal muscular atrophy due to homozygous deletion of exon 7 in SMN1

From: https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp/index.html

PRESYMPTOMATIC GENETIC TESTING

In addition to testing for genetic diseases in fetuses and newborns, testing of healthy adults for genes that might predispose them to a genetic condition in the future is possible. This type of testing is known as **presymptomatic genetic testing**. For example, presymptomatic testing is available for members of families in which an autosomal dominant form of breast cancer occurs. In this case, early identification of the disease-causing allele allows for closer surveillance and the early detection of tumors.

Presymptomatic testing is also available for some genetic diseases for which no treatment is available, such as Huntington disease, an autosomal dominant disease that leads to slow physical and mental deterioration in middle age.

HETEROZYGOTE SCREENING

Another form of genetic testing in adults is **heterozygote screening**. In this type of screening, members of a population are tested to identify heterozygous carriers of recessive disease-causing alleles—people who are healthy but have the potential to produce children with a particular disease.

Testing for Tay–Sachs disease is a successful example of heterozygote screening. In the general population of North America, the frequency of Tay–Sachs disease is only about 1 person in 360,000. Among Ashkenazi Jews (descendants of Jewish people who settled in eastern and central Europe), the frequency is 100 times greater. A simple blood test is used to identify Ashkenazi Jews who carry the allele for Tay–Sachs disease. If a man and woman are both heterozygotes, approximately one-fourth of their children are expected to have Tay–Sachs disease. Couples identified as heterozygous carriers may use that information in deciding whether to have children. A prenatal test is also available for determining if the fetus of an at-risk couple will have Tay–Sachs disease. Screening programs have led to a significant decline in the number of children

of Ashkenazi ancestry born with Tay–Sachs disease (now fewer than 10 children per year in the United States).

PHARMACOGENETIC TESTING

Genetic testing is becoming increasingly important in guiding drug treatment. In some cases, a drug's effectiveness is influenced by a patient's genotype; in other cases, individuals with certain genotypes may be more likely to suffer from adverse drug reactions.

Warfarin provides a good example of how genetic testing can be used in conjunction with drug treatment. Warfarin is the most common anticoagulant (blood thinner) used worldwide. It is often administered to people with certain heart problems and after surgery to prevent the formation of clots in the blood vessels, which can obstruct blood flow and lead to tissue death. An effective dose of warfarin is critical: too little, and blood clots are not prevented; too much, and internal bleeding results. Unfortunately, people vary greatly in their responses to warfarin, and some of this variation is due to genes. For example, *CYP2C9* is a gene that encodes an enzyme that metabolizes warfarin. Over 30 different alleles occur at this locus. People who are homozygous for the *CYP2C9*1* allele metabolize warfarin normally, but individuals who are homozygous or heterozygous for the *CYP2CP9*2* or *CYP2CP9*3* alleles metabolize warfarin at a much lower rate and therefore require a lower dose. If given the usual dose of warfarin, these people are at greater risk of

bleeding. Genetic variation at *CYP2CP* and another locus called *VKORC1* accounts for up to 30% of the variation in response to warfarin dose and risk of bleeding. Some hospitals are screening patients for variation at these genes to help determine the proper warfarin dose to administer. A study conducted by Vanderbilt University in Nashville, Tennessee, found that over 90% of all patients carried one or more genetic variants that affected their response to five common drugs, including warfarin. **› TRY PROBLEM 12**

THINK-PAIR-SHARE Questions 7 and 8

CONCEPTS

Genetic testing is used to screen newborns for genetic diseases, detect individuals who are heterozygous for recessive diseases, detect disease-causing alleles in those who have not yet developed symptoms of a disease, and detect defective alleles in unborn babies. Preimplantation genetic diagnosis combined with in vitro fertilization allows for the selection of embryos that are free from specific genetic diseases. Genetic testing is also being used to guide individual drug treatment.

✔ CONCEPT CHECK 9

How does preimplantation genetic diagnosis differ from prenatal genetic testing?

Interpreting Genetic Tests

Today, more than a thousand genetic tests are clinically available, and several hundred more are available through research studies. Future research will greatly increase the number and complexity of genetic tests. Many of these tests will be for complex multifactorial diseases that are influenced by both genetics and environment, such as coronary artery disease, diabetes, asthma, some types of cancer, and depression.

Interpreting the results of genetic tests is often complicated by several factors. First, some genetic diseases can be caused by many different mutations. For example, more than a thousand different mutations at a single locus can cause cystic fibrosis, an autosomal recessive disease in which chloride ion transport is defective (see Chapter 5). Genetic tests typically screen for only the most common mutations; uncommon and rare mutations are not detected. Therefore, a negative result does not mean that a genetic defect is absent; it indicates only that the person being tested does not have one of the common mutations. The DNA of an affected person can be examined to determine the nature of the mutation, and other family members can then be screened for the same mutation, but this option is not possible if affected family members are unavailable or unwilling to be tested.

A second problem lies in interpreting the results of genetic tests. For a classic genetic disease such as Tay–Sachs disease, the inheritance of two copies of the gene virtually ensures that a person will have the disease. However, this is not the case for many genetic diseases

for which penetrance is incomplete and environmental factors play a role. For these conditions, carrying a disease-predisposing mutation only elevates a person's risk of acquiring the disease. The risk associated with a particular mutation is a statistical estimate, based on the average effect of the mutation on many people. In this case, the calculated risk may provide little useful information to a specific person. It is also important to keep in mind that for many genetic traits and disorders, no genetic test exists.

CONCEPTS

Interpreting genetic tests is complicated by the presence of multiple causative mutations, incomplete penetrance, and the influence of environmental factors.

Direct-to-Consumer Genetic Testing

Genome sequencing and new molecular technologies now make it possible to determine a person's risk of a large number of diseases and disorders. **Direct-to-consumer genetic tests** attempt to make this information available to any consumer, without involving a health-care provider. Several companies sold such tests in the United States prior to 2013, when their sale was restricted by the U.S. Food and Drug Administration (FDA). In 2017, the FDA began allowing direct-to-consumer genetic tests that provide information on predisposition to certain diseases. These tests are potentially

capable of screening for a large array of genetic conditions in adults and children—everything from single-gene disorders such as cystic fibrosis to multifactorial conditions such as obesity, cardiovascular disease, potential for athletic performance, and predisposition to nicotine addiction.

Most direct-to-consumer genetic tests are advertised and ordered through the Internet. Geneticists, public-health officials, and consumer advocates have raised a number of concerns about direct-to-consumer genetic testing, including concerns that some tests are offered without appropriate information and genetic counseling and that consumers are often not equipped to interpret the results. Direct-to-consumer tests are also available for paternity testing and for determining ancestry (see next section). › **TRY** PROBLEM 15

THINK-PAIR-SHARE Question 9

Genealogies and Genetic Testing for Ancestry

Many people are curious about their ancestry. A number of Web sites facilitate the collection of genealogical records, with over 100 million users in 2018. Some of these genealogical databases automatically detect similarities between family trees and offer the option to merge family records from different sources, creating very

large groups with thousands or even millions of individuals connected by shared ancestry. In addition, DNA tests are available to help people study their ancestry. For example, the Genographic Project, sponsored by National Geographic, determines ancient ancestry based on DNA testing; it provides information on regional ancestry—the proportion of one's genome that comes from nine ancestral source populations—as well as the presence of any Neanderthal DNA in one's genome. Other databases compile data from genetic testing to help people find their biological relatives.

Researchers have begun to use genealogical and ancestry databases for examining questions about human genetics and evolution, including the inheritance of traits, the genetic structure of populations, migration, and changing patterns of relatedness and fertility. For example, researchers examined over 60,000 participants of direct-to-consumer genetic testing to study the genetics of earlobe attachment and demonstrated that—contrary to common wisdom—free versus attached earlobes are not inherited as a simple genetic trait; rather, this trait is polygenic and determined by variation at a number of different genes. The use of these databases for research and other purposes has, however, raised ethical concerns about using the information in ways that were not anticipated by the participants.

Genetic Discrimination and Privacy

With the development of so many new genetic tests, concerns have been raised about the privacy of genetic information and the potential for genetic discrimination. Research shows that many people at risk for genetic diseases avoid genetic testing because they fear that the results would make it difficult for them to obtain insurance or that the information might adversely affect their employability. Some of those who do seek genetic testing pay for it themselves and use aliases to prevent the results from becoming part of their health records. Fears about genetic discrimination have been reinforced by past practices. In the 1970s, some people who were identified as carriers of sickle-cell anemia (an autosomal recessive disorder that is common among African Americans) faced employment discrimination and had difficulty obtaining health insurance, in spite of the fact that carriers are healthy.

In response to these concerns, the U.S. Congress passed the **Genetic Information Nondiscrimination Act (GINA)** in 2008. This law prohibits health insurers from using genetic information to make decisions about health-insurance coverage and rates. It also prevents employers from using genetic information in employment decisions and prohibits health insurers and employers from asking or requiring a person to take a genetic test. Results of genetic testing receive some degree of protection by other federal regulations that cover the uses and disclosure of individual health information. However, GINA covers health insurance and employment only; it does not apply to life, disability, and long-term care insurance. › **TRY PROBLEM 16**

CONCEPTS

The growing number of genetic tests and their increasing complexity have raised several concerns, including concerns about genetic discrimination and the privacy of test results.

Chapter 6 Review

CHAPTER SUMMARY

Section 6.1 Learning Objective: Be able to explain why pedigrees are often used in the study of human genetics.

- Constraints on the genetic study of human traits include the inability to conduct controlled crosses, long generation time, small family size, and the difficulty of separating genetic and environmental influences. Pedigrees are often used to study the inheritance of traits in humans.

Section 6.2 Learning Objective: Be able to use pedigrees to analyze the genetic basis of traits in humans.

- Special symbols and formats are used to construct pedigrees.
- Autosomal recessive traits typically appear with equal frequency in both sexes and tend to skip generations. When both parents are heterozygous for a particular autosomal recessive trait, approximately one-fourth of their offspring will have the trait. Recessive traits are more likely to appear in families with consanguinity (mating between closely related people).

- Autosomal dominant traits usually appear equally in both sexes and do not skip generations. When one parent is affected and heterozygous for an autosomal dominant trait, approximately half of the offspring will have the trait. Unaffected people do not normally transmit an autosomal dominant trait to their offspring.
- X-linked recessive traits appear more frequently in males than in females. When a woman is a heterozygous carrier for an X-linked recessive trait and a man is unaffected, approximately half of their sons will have the trait and half of their daughters will be unaffected carriers. X-linked traits are not passed from father to son.
- X-linked dominant traits appear in both males and females but more frequently in females. They do not skip generations. Affected men pass an X-linked dominant trait to all their daughters but none of their sons. Heterozygous women pass the trait to half of their sons and half of their daughters.
- Y-linked traits appear only in males and are passed from a father to all his sons.

Section 6.3 Learning Objective: Be able to explain how twins and adoption studies are used to assess the role of genetic and environmental factors in human traits.

- A trait's higher concordance in monozygotic than in dizygotic twins indicates a genetic influence on the trait; less than 100% concordance in monozygotic twins indicates environmental influences on the trait.

- Similarities between adopted children and their biological parents indicate the importance of genetic factors in the expression of a trait; similarities between adopted children and their genetically unrelated adoptive parents indicate the influence of environmental factors.

Section 6.4 Learning Objective: Be able to describe genetic counseling and explain how genetic testing is used to help people concerned about genetic traits.

- Genetic counseling provides information and support to people concerned about hereditary conditions in their families.
- Genetic testing includes prenatal diagnosis, screening for disease-causing alleles in newborns, the detection of people heterozygous for recessive alleles, presymptomatic testing for the presence of a disease-causing allele in at-risk people, and testing to evaluate the effectiveness and safety of drug treatment.
- The interpretation of genetic tests may be complicated by the presence of numerous causative mutations, incomplete penetrance, and the influence of environmental factors.
- The availability of direct-to-consumer genetic tests has raised concerns about the adequacy of the information provided and the absence of genetic counseling. Genealogical Web sites and genetic tests provide information about ancestry and are sometimes used to find biological relatives.
- Genetic testing has raised concerns about genetic discrimination and the privacy of test results. The Genetic Information Nondiscrimination Act prohibits the use of genetic

information in decisions on health insurability and employment.

WORKED PROBLEMS

Problem 1

Joanna has "short fingers" (brachydactyly). She has two older brothers who are identical twins; both have short fingers. Joanna's two younger sisters have normal fingers. Joanna's mother has normal fingers, and her father has short fingers. Joanna's paternal grandmother (her father's mother) has short fingers; her paternal grandfather (her father's father), who is now deceased, had normal fingers. Both of Joanna's maternal grandparents (her mother's parents) have normal fingers. Joanna marries Tom, who has normal fingers; they adopt a son named Bill, who has normal fingers. Bill's biological parents both have normal fingers. After adopting Bill, Joanna and Tom produce two children: an older daughter with short fingers and a younger son with normal fingers.

a. Using standard symbols and labels, draw a pedigree illustrating the inheritance of short fingers in Joanna's family.
b. What is the most likely mode of inheritance for short fingers in this family?
c. If Joanna and Tom have another biological child, what is the probability (based on your answer to part *b*) that this child will have short fingers?

» Solution Strategy

What information is required in your answer to the problem?

a. A pedigree to represent the family, drawn with correct symbols and labeling.
b. The most likely mode of inheritance for short fingers.
c. The probability that Joanna and Tom's next child will have short fingers.

What information is provided to solve the problem?

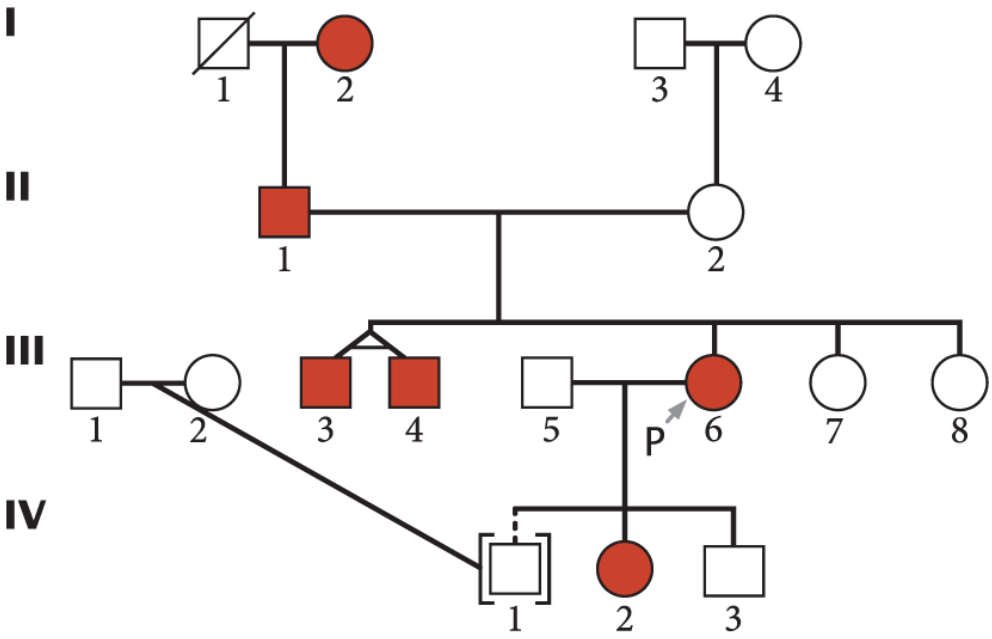
The phenotypes of Joanna and Tom and their family members.

For help with this problem, review:

The information on pedigrees in Section 6.2.

» Solution Steps

a. In the pedigree for the family, use filled circles (females) and filled squares (males) to represent family members with the trait of interest (short fingers). Connect Joanna's identical twin brothers to the line above by drawing diagonal lines that have a horizontal line between them. Enclose Bill, the adopted child of Joanna and Tom, in brackets; connect him to his biological parents by drawing a diagonal line and to his adoptive parents by a dashed line.



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Description

Generation 1 shows two couples, couple 1 has an unaffected male crossed out and an affected female. Couple 2 has both unaffected male and female. Couple 1 mates and has an affected son in generation 2. Couple 2 mates and has an unaffected daughter. Both these offspring in generation 2 mate and have 1 affected daughter who is a proband, 2 unaffected daughter, and a pair of affected identical twin sons in generation 3. Another couple in generation 3 has unaffected male and unaffected female. They mate and have an unaffected son in generation 4. The proband female from generation 3 mates with an unaffected male and has 1 affected daughter, 1 unaffected son and adopts the unaffected son of the unaffected couple from generation 3.

Hint: See Figure 6.2 for a review of symbols used in a pedigree.

b. The most likely mode of inheritance for short fingers in this family is autosomal dominant. The trait appears equally in

males and females and does not skip generations. When one parent has the trait, it appears in approximately half of that parent's sons and daughters, although the number of children in the families is small. We can eliminate Y-linked inheritance because the trait is found in females as well as males. If short fingers were X-linked recessive, females with the trait would be expected to pass the trait to all their sons, but Joanna (III-6), who has short fingers, produced a son with normal fingers. For X-linked dominant traits, affected men should pass the trait to all their daughters; because male II-1 has short fingers and produced two daughters without short fingers (III-7 and III-8), we know that the trait cannot be X-linked dominant. The trait is unlikely to be autosomal recessive because it does not skip generations and because approximately half the children of affected parents have the trait.

See Table 6.1 for a review of the characteristics of different modes of inheritance.

c. If having short fingers is an autosomal dominant trait, Tom must be homozygous (*bb*) because he has normal fingers. Joanna must be heterozygous (*Bb*) because she and Tom have produced both short-fingered and normal-fingered offspring. In a cross between a heterozygote and a homozygote, half the progeny are expected to be heterozygous and the other half homozygous ($Bb \times bb \rightarrow {}^1/_2Bb, {}^1/_2bb$), so the probability that Joanna and Tom's next biological child will have short fingers is ${}^1/_2$.

Problem 2

Concordance for a series of traits was measured in monozygotic twins and dizygotic twins; the results are shown in the following table. For each trait, indicate whether the concordances suggest genetic influences, environmental influences, or both. Explain your reasoning.

		Concordance (%)	
	Characteristic	**Monozygotic**	**Dizygotic**
a.	ABO blood type	100	65
b.	Diabetes	85	36
c.	Coffee drinking	80	80
d.	Smoking	75	42
e.	Schizophrenia	53	16

» Solution Strategy

What information is required in your answer to the problem?

For each trait, whether it is influenced by genetic factors, environmental factors, or both.

What information is provided to solve the problem?

Concordance for each trait in monozygotic and dizygotic twins.

For help with this problem, review:

Concordance in Twins in Section 6.3.

» Solution Steps

a. The concordance for ABO blood type in monozygotic twins is 100%. This high concordance in monozygotic twins does not, by itself, indicate a genetic basis for the trait. Because concordance for ABO blood type is substantially lower in dizygotic twins, we would be safe in concluding that genes play a role in determining differences in ABO blood types.

> **Recall:** The influence of genetic factors on the trait is indicated by higher concordance in monozygotic twins than dizygotic twins.

b. The concordance for diabetes is substantially higher in monozygotic twins than in dizygotic twins; therefore, we can conclude that genetic factors play some role in susceptibility to diabetes. The fact that monozygotic twins show a concordance less than 100% suggests that environmental factors also play a role.

> **Recall:** Less than 100% concordance in monozygotic twins indicates that environmental factors play a role in the trait.

c. Both monozygotic and dizygotic twins exhibit the same high concordance for coffee drinking, so we can conclude that there

is little genetic influence on coffee drinking. The fact that monozygotic twins show a concordance less than 100% suggests that environmental factors play a role.

d. The concordance for smoking is lower in dizygotic twins than in monozygotic twins, so genetic factors appear to influence the tendency to smoke. The fact that monozygotic twins show a concordance less than 100% suggests that environmental factors also play a role.
e. Monozygotic twins exhibit substantially higher concordance for schizophrenia than do dizygotic twins, so we can conclude that genetic factors influence this psychiatric disorder. Because the concordance of monozygotic twins is substantially less than 100%, we can conclude that environmental factors play a role in the disorder as well.

IMPORTANT TERMS

pedigree
proband
consanguinity
genetic mosaic
dizygotic twins
monozygotic twins
concordance
genetic counseling
ultrasonography
amniocentesis

chorionic villus sampling (CVS)
karyotype
maternal blood screening test
noninvasive prenatal genetic screening (NIPS)
preimplantation genetic diagnosis (PGD)
newborn screening
presymptomatic genetic testing
heterozygote screening
direct-to-consumer genetic test
Genetic Information Nondiscrimination Act (GINA)

ANSWERS TO CONCEPT CHECKS

1. b
2. It might skip generations when a new mutation arises or the trait has incomplete penetrance.
3. If it is X-linked recessive, the trait will not be passed from father to son.
4. c
5. If the trait were Y linked, an affected male would pass it on to all his sons, whereas if the trait were autosomal and sex-limited, affected heterozygous males would pass it on to only half of their sons on average.
6. d
7. c
8. d

9. Preimplantation genetic diagnosis determines the presence of disease-causing genes in an embryo at an early stage, before the embryo is implanted in the uterus and initiates pregnancy. Prenatal genetic testing determines the presence of disease-causing genes or chromosomes in a developing fetus.

Chapter 6 Assessment

COMPREHENSION QUESTIONS

Section 6.1

1. What three factors complicate the task of studying the inheritance of human characteristics?

Section 6.2

2. Who is the proband in a pedigree? Is the proband always found in the last generation of the pedigree? Why or why not?

3. For each of the following modes of inheritance, describe the features that will be exhibited in a pedigree in which the trait is present: autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant, and Y-linked inheritance.

4. How does the pedigree of an autosomal recessive trait differ from the pedigree of an X-linked recessive trait?

5. Other than the fact that a Y-linked trait appears only in males, how does the pedigree of a Y-linked trait differ from the pedigree of an autosomal dominant trait?

Section 6.3

6. What are the two types of twins, and how do they arise?

7. Explain how a comparison of concordance in monozygotic and dizygotic twins can be used to determine the extent to which the expression of a trait is influenced by genes or by environmental factors.

8. How are adoption studies used to separate the effects of genes and environment in the study of human characteristics?

Section 6.4

9. What is genetic counseling?

10. *Give at least four different reasons for seeking genetic counseling.

11. Briefly define newborn screening, heterozygote screening, presymptomatic genetic testing, and prenatal diagnosis.

12. *Compare the advantages and disadvantages of amniocentesis versus chorionic villus sampling for prenatal diagnosis.

13. What is preimplantation genetic diagnosis?

14. How does heterozygote screening differ from presymptomatic genetic testing?

15. *What are direct-to-consumer genetic tests? What are some of the concerns about these tests?

16. *What activities does the Genetic Information Nondiscrimination Act prohibit?

17. How might genetic testing lead to genetic discrimination?

APPLICATION QUESTIONS AND PROBLEMS

Section 6.1

18. *If humans have characteristics that make them unsuitable for genetic analysis, such as long generation time, small family size, and uncontrolled crosses, why do geneticists study humans? Give several reasons why humans have been the focus of so much genetic study.

Section 6.2

19. *Joe is color blind. Both his mother and his father have normal color vision, but his mother's father (Joe's maternal grandfather) is color blind. All Joe's other grandparents have normal color vision. Joe has three sisters—Patty, Betsy, and Lora—all with normal color vision. Joe's oldest sister, Patty, is married to a man with normal color vision; they have two children: a 9-year-old color-blind boy and a 4-year-old girl with normal color vision.

a. Using standard symbols and labels, draw a pedigree of Joe's family.

b. What is the most likely mode of inheritance for color blindness in Joe's family?

c. If Joe marries a woman who has no family history of color blindness, what is the probability that their first child will be a color-blind boy?

d. If Joe marries a woman who is a carrier of the color-blind allele, what is the probability that their first child will be a color-blind boy?

e. If Patty and her husband have another child, what is the probability that the child will be a color-blind boy?

20. Consider the pedigree shown in **Figure 6.3a**.

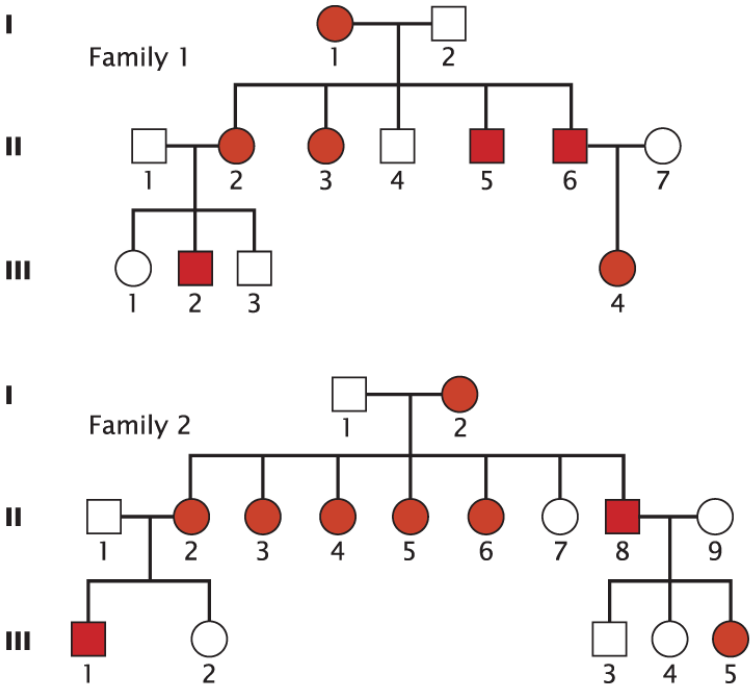
a. If individual IV-7 married a person who was unaffected with Waardenburg syndrome, what is the probability that their first child would have Waardenburg syndrome? Explain your reasoning.

b. If individuals IV-4 and IV-5 mated and produced a child, what is the probability that the child would have Waardenburg syndrome? Explain your reasoning.

21.

Many studies have suggested a strong genetic predisposition to migraine headaches, but the mode of inheritance is not clear. L. Russo and colleagues examined

migraine headaches in several families, two of which are shown below. What is the most likely mode of inheritance for migraine headaches in these families? Explain your reasoning.



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(Data from L. Russo et al. 2005. *American Journal of Human Genetics* 76:327–333)

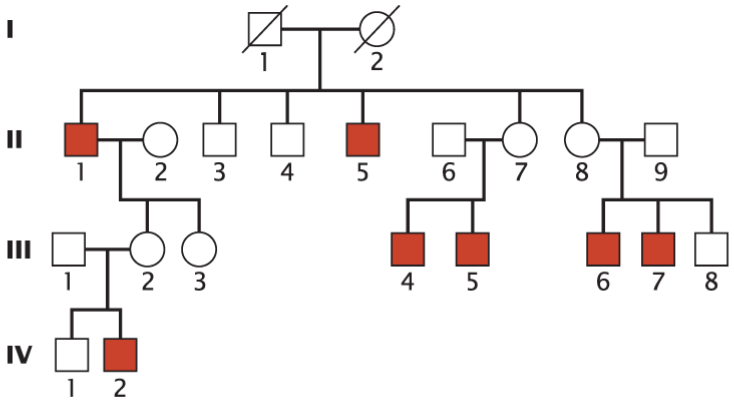
Description

In the case of first family shown at the top: in generation 1, the female is affected and passes the trait on to 4 of 5 children. Half of the affected children are boys and half are girls. Only one affected male and female have children. The female has three children, one of which, a male, is affected with the trait. For the other child in generation 2, he has one female child who is also affected with the trait. In the case of second family shown below: in generation 1, the female is affected and passes the trait on to 6 of 7 children. Six of 7 children are girls. Five of the girls and the one boy are affected with the trait. Only one affected male and female have children. The female has two children, one of which, a male, is affected. For the other male child in generation 2, he has three children, and one, a female displays the trait.

22. DATA ANALYSIS *Dent disease is a rare disorder of the kidney in which reabsorption of filtered solutes is impaired and there is progressive renal failure. R. R. Hoopes and colleagues studied mutations associated with Dent disease in the following family:

a. On the basis of this pedigree, what is the most likely mode of inheritance for the disease? Explain your reasoning.

b. Based on your answer to part *a*, give the most likely genotypes for all family members in the pedigree.



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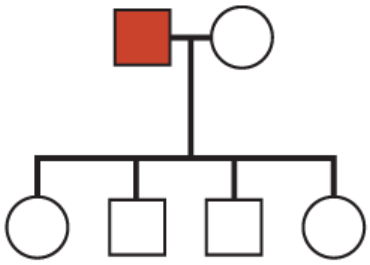
(Data from R. R. Hoopes et al. 2005. *American Journal of Human Genetics* 76:260–267)

Description
Generation 1 has 2 unaffected parents crossed out. They had 6 offspring: 2 positive males, 2 negative males, and 2 negative females. Of these offspring, 1 positive male and 2 negative females mated and had offspring. The male had 2 female offspring negative for the trait and 1 out of 2 grandsons positive for the disorder. The 2 negative females had 5 sons, 4 of which were positive for the disorder. They had no female offspring.

23. CONNECT Read again the introductory story for Chapter 3, The Genetics of Blond Hair in the South Pacific. Based on the information provided in this story, draw a pedigree of a typical Solomon Islander family in which blond hair occurs. Be sure to incorporate the mode of inheritance for blond hair in Pacific Islanders and correct symbols for a pedigree.

24. A man with a specific unusual genetic trait marries an unaffected woman and they have four children. Pedigrees of this family are shown in parts *a* through *e*, but the presence or absence of the trait in the children is not indicated. For each type of inheritance, indicate how many children of each sex are expected to express the trait by filling in the appropriate circles and squares. Assume that the trait is rare and fully penetrant.

a. Autosomal recessive trait



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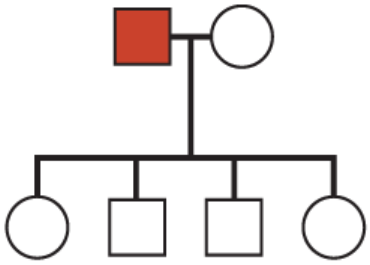
Description

The pedigree chart shows a couple in generation 1 where male is affected and female is unaffected. The couple has 2 unaffected daughters and 2 unaffected sons in generation 2.

b. Autosomal dominant trait



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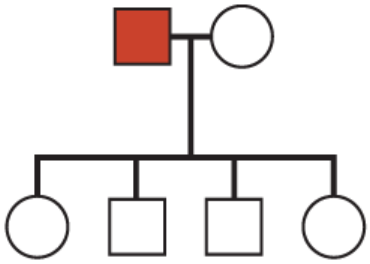
Description
The pedigree chart shows a couple in generation 1 where male is affected and female is unaffected. The couple has 2 unaffected daughters and 2 unaffected sons in generation 2.

c. X-linked recessive trait



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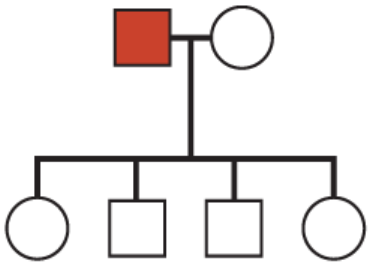
Description
The pedigree chart shows a couple in generation 1 where male is affected and female is unaffected. The couple has 2 unaffected daughters and 2 unaffected sons in generation 2.

d. X-linked dominant trait



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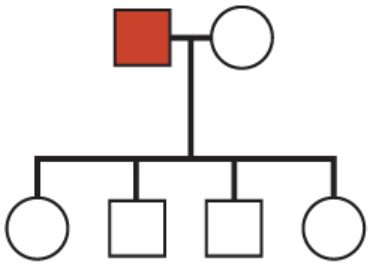
Description
The pedigree chart shows a couple in generation 1 where male is affected and female is unaffected. The couple has 2 unaffected daughters and 2 unaffected sons in generation 2.

e. Y-linked trait



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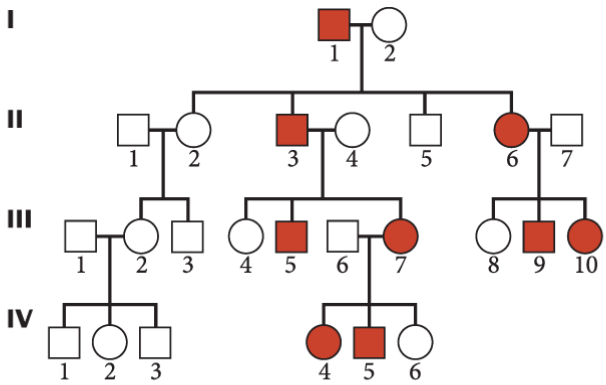
Description
The pedigree chart shows a couple in generation 1 where male is affected and female is unaffected. The couple has 2 unaffected daughters and 2 unaffected sons in generation 2.

25. *For each of the following pedigrees, give the most likely mode of inheritance, assuming that the trait is rare. Carefully explain your reasoning.

a.



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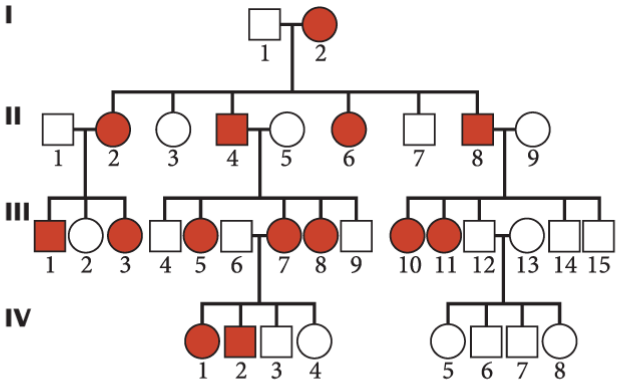
Description
In generation 1, the male is affected. They have 2 sons and 2 daughters. Of these, one son and one daughter are affected. The unaffected daughter has 2 kids and one of her kids has 3 kids. None of the people display the trait. The affected male and female from generation 2 both have offspring and pass down the trait. The male has 2 girls and one son. Of these, one male and female display the trait. The daughter from generation 3 has 3 offspring, of which one son and one daughter display the trait. The male offspring from generation 2 has 3 children (2 daughters and 1 son), of which one male and one female display the trait.

b.



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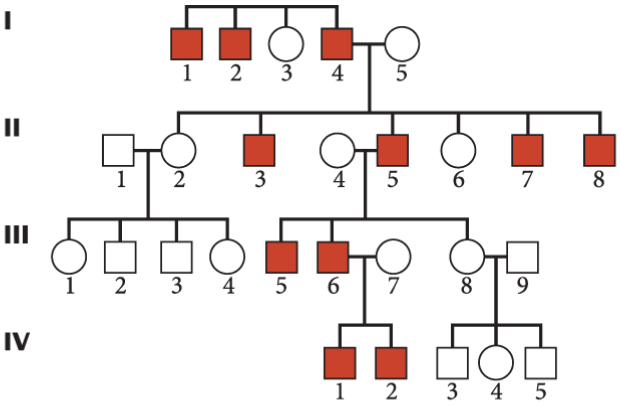
Description

In generation 1, the female is affected. The couple has 6 offspring: 2 positive females, 1 negative female, 2 positive males, and 1 negative male. Of these offspring, 1 positive female and 2 positive males mate and have offspring. The positive female mates a negative male, and they have 1 positive male, 1 negative female, and one positive female. Both positive males from generation 2 mate a negative partner, and have 10 offspring: 5 negative males and 5 positive females. Of these offspring, one negative male and one positive female mate and have offspring. The male has 2 negative male and 2 negative female offspring. The positive female mates a negative male, and they have one positive male, one positive female, one negative male, and one negative female offspring.

c.



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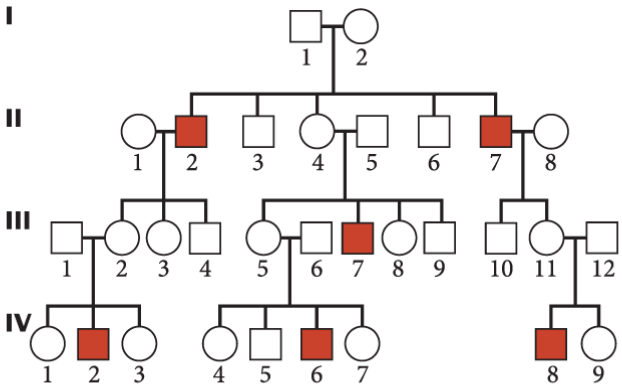
Description

In generation 1, there are 4 siblings, 3 positive brothers and one negative sister. Of these siblings, only one positive male mates and has offspring. The female parent is negative for the trait. They have 4 positive male and 2 negative female offspring. Of the 6, one negative female and one positive male mate negative partners and have offspring. The female has no positive offspring. The male has 2 positive male and one negative female offspring. One male and one female mate and have offspring. The one male has 2 male offspring, both of which are positive for the trait. The female has 2 male and 1 female offspring, all of which are negative for the trait.

d.



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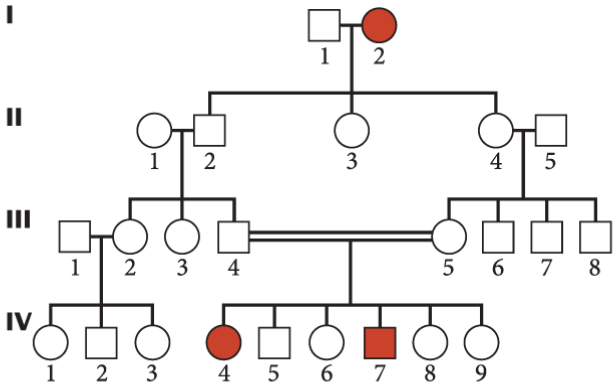
Description

In generation 1, the male and female parents are both negative. They have 5 offspring: 2 positive males, 2 negative males, and one negative female. 3 of these offspring mate negative partners and have offspring. Of the positive males, they have 3 negative female and 2 negative male offspring. Two of these females mate negative partners, and they have 3 negative females and 2 positive male offspring. In generation 2, the negative female gives birth to 2 negative females, 1 positive male, and one negative male offspring. Only one negative female mates a negative partner and has children. She has 2 negative females, 1 negative male, and 1 positive male offspring.

e.



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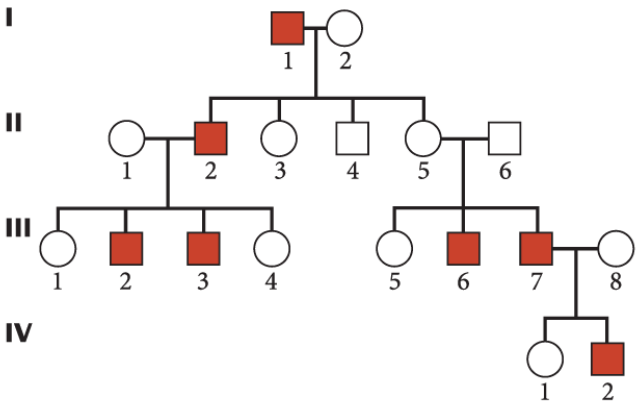
Description

Generation 1 has a couple where male is unaffected and female is affected. The couple has 1 unaffected son and 2 unaffected daughters in generation 2. The unaffected male from generation 2 mates with an unaffected female and has 2 unaffected daughters and 1 unaffected son. One unaffected female from generation 2 mates with an unaffected male and has 3 unaffected sons and 1 unaffected daughter. One unaffected daughter of the unaffected male from generation 2 mates with an unaffected male and has 2 unaffected daughters and 1 unaffected son in generation 4. The unaffected son of the unaffected male from generation 2 mates with the unaffected daughter of the unaffected female from generation 2. This is a consanguineous mating between cousins. They have 1 affected daughter, 1 affected son, 1 unaffected son, and 3 unaffected daughters in generation 4.

26. The trait represented in the following pedigree is expressed only in the males of the family. Is the trait Y linked? Why or why not? If you believe that the trait is not Y linked, propose an alternative explanation for its inheritance.



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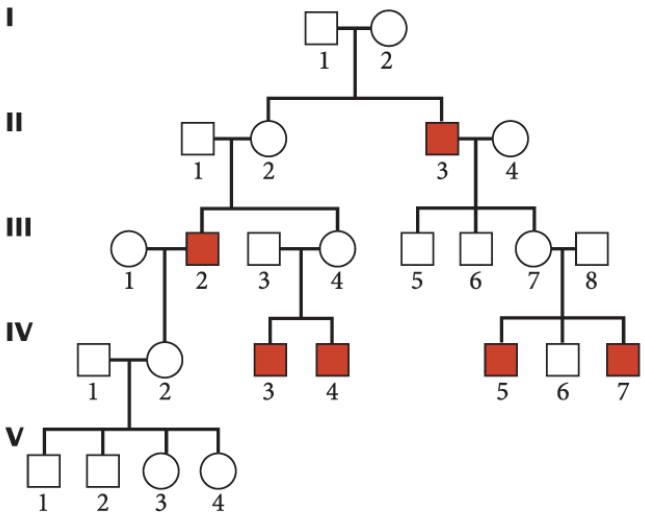
Description

The pedigree chart shows generation 1 where male is positive and female is negative. They have 4 offsprings: 1 positive male, 1 negative male, and 2 negative females in generation 2. Of these offspring, one positive male and one negative female mate negative partners and have offspring. The positive male has 2 positive male and 2 negative female offspring. The negative female from generation 2 has 1 negative female and 2 positive male offspring. One of these positive males mates and has one negative female and one positive male offspring.

27. DATA ANALYSIS The following pedigree illustrates the inheritance of Nance–Horan syndrome, a rare genetic condition in which affected people have cataracts and abnormally shaped teeth.



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[Data from D. Stambolian et al., 1990. *American Journal of Human Genetics* 47:15.]

Description

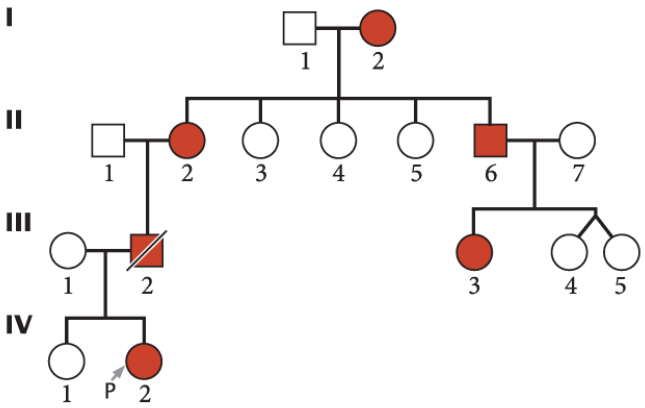
Generation 1 has a couple where the male and female are both unaffected. The couple has 1 unaffected daughter and 1 affected son in generation 2. The unaffected female from generation 2 mates with an unaffected male and has 1 unaffected daughter and 1 affected son in generation 3. The affected male from generation 2 mates with an unaffected female and has 1 unaffected daughter and 2 unaffected sons in generation 3. The affected male from generation 3 mates with an unaffected female and has 1 unaffected daughter in generation 4. Unaffected daughter of unaffected female from generation 2 mates with an unaffected male and has 1 affected daughter and 1 affected son in generation 4. Unaffected daughter of affected male from generation 2 mated with an unaffected male and has 2 affected sons and 1 unaffected son in generation 4. Unaffected daughter of affected male from generation 3 mates with an unaffected male and has 2 unaffected daughters and 2 unaffected sons in generation 5.

a. On the basis of this pedigree, what do you think is the most likely mode of inheritance for Nance–Horan syndrome?

b. If couple III-7 and III-8 have another child, what is the probability that the child will have Nance–Horan syndrome?

c. If III-2 and III-7 were to mate, what is the probability that one of their children would have Nance–Horan syndrome?

28. DATA ANALYSIS The following pedigree illustrates the inheritance of ringed hair, a condition in which each hair is differentiated into light and dark zones. What mode or modes of inheritance are possible for the ringed-hair trait in this family?



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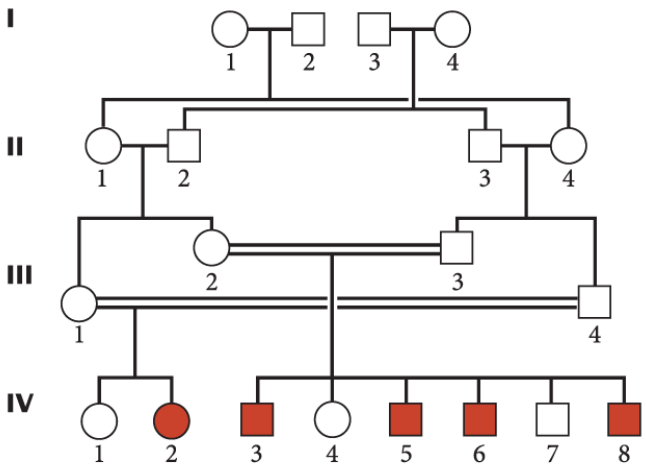
[Data from L. M. Ashley and R. S. Jacques. 1950. *Journal of Heredity* 41:83.]

Description

Generation 1 has a couple where there is an affected female and an unaffected male. They have 5 offspring: 1 affected son, 1 affected daughter, and 3 unaffected daughters in generation 2. The affected female from generation 2 mates with an unaffected male and has an affected son is crossed out in generation 3. The affected male from generation 2 mates with an unaffected female and has 1 affected daughter and a pair of non-identical unaffected twin daughters in generation 3. The

affected male crossed out from generation 3 mates with an unaffected female and has 1 affected daughter who is a proband and 1 unaffected daughter in generation 4.

29. DATA ANALYSIS Ectrodactyly is a rare condition in which the fingers are absent and the hand is split. This condition is usually inherited as an autosomal dominant trait. Ademar Freire-Maia reported the appearance of ectrodactyly in a family in São Paulo, Brazil, whose pedigree is shown here. Is this pedigree consistent with autosomal dominant inheritance? If not, what mode of inheritance is most likely? Explain your reasoning.



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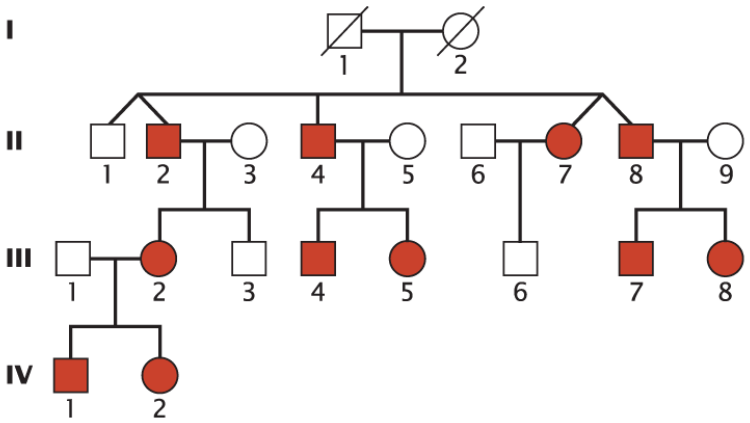
[Data from A. Freire-Maia. 1971. *Journal of Heredity* 62:53.]

Description

Generation 1 shows two couples where males and females are unaffected in both the couples. Couple 1 has 2 unaffected daughters in generation 2 and couple 2 has 2 unaffected sons in generation 2. One daughter of couple 1 mates with a son of couple 2. They have 2 unaffected daughters in generation 3. The other daughter of couple 1 mates with the other son of couple 2 and has 2 unaffected sons in

generation 3. One unaffected female from generation 3 mates with 1 unaffected male from generation 3. This is consanguineous mating and has 1 unaffected daughter, 4 affected sons, and 1 unaffected son in generation 4. Another unaffected female from generation 3 mates with another unaffected male from generation 3 which is also a consanguineous mating. They have 1 affected daughter and 1 unaffected daughter in generation 4.

30. DATA ANALYSIS The complete absence of one or more teeth (tooth agenesis) is a common trait in humans—indeed, more than 20% of humans lack one or more of their third molars. However, more severe tooth agenesis, defined as the absence of six or more teeth, is less common and is frequently an inherited condition. L. Lammi and colleagues examined tooth agenesis in the Finnish family shown in the pedigree below.



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[Data from L. Lammi et al., 2004. *American Journal of Human Genetics* 74:1043–1050.]

Description
Generation 1 has a couple with an unaffected male crossed out and an unaffected female crossed out. They have 2 pairs of non-identical twins and 1 affected son in

generation 2. First pair of twins has 1 unaffected son and 1 affected son. Second pair of twins has 1 affected daughter and 1 affected son. The affected son from first twin pair mates with an unaffected female and has 1 affected daughter and 1 unaffected son in generation 3. The affected son who is a singleton mates with an unaffected female and has 1 affected son and 1 affected daughter in generation 3. The affected female from second pair of twins mates with an unaffected male and has 1 unaffected son in generation 3. The affected male from second pair of twins mates with an unaffected female and has 1 affected son and 1 affected daughter in generation 3. The affected daughter of the affected male from first pair of twins mates with an unaffected male and has 1 affected daughter and 1 affected son in generation 4.

a. What is the most likely mode of inheritance for tooth agenesis in this family? Explain your reasoning.

b. Are the two sets of twins in this family monozygotic or dizygotic twins? What is the basis of your answer?

c. If IV-2 married a man who had a full set of teeth, what is the probability that their child would have tooth agenesis?

d. If III-2 and III-7 married and had a child, what is the probability that their child would have tooth agenesis?

31. CONNECT How did our approach in Chapter 3 to determine whether a trait is recessive or dominant differ from our use of pedigrees in this chapter to determine whether a trait is recessive or dominant?

Section 6.3

32. *A geneticist studies a series of characteristics in pairs of monozygotic and dizygotic twins, obtaining the following concordances. For each characteristic, indicate whether these concordances suggest genetic influences, environmental influences, or both. Explain your reasoning.

	Concordance (%)	
Characteristic	**Monozygotic**	**Dizygotic**
Migraine headaches	60	30
Eye color	100	40
Measles	90	90
Club foot	30	10
High blood pressure	70	40
Handedness	70	70
Tuberculosis	5	5

33. On the basis of the concordances shown in Table 6.2, is variation in rheumatoid arthritis influenced by genetic factors, environmental factors, or both? Explain your reasoning.

34. DATA ANALYSIS M. T. Tsuang and colleagues studied drug dependence in male twin pairs (M. T. Tsuang et al. 1996. *American Journal of*

Medical Genetics 67:473–477). They found that 82 out of 313 monozygotic twin pairs were concordant for abuse of one or more illicit drugs, whereas 40 out of 243 dizygotic twin pairs were concordant for the same trait. Calculate the concordances for drug abuse in these monozygotic and dizygotic twins. On the basis of these data, what conclusion can you draw concerning the roles of genetic and environmental factors in drug abuse?

35.

*In a study of schizophrenia (a mental disorder including disorganization of thought and withdrawal from reality), researchers looked at the prevalence of the disorder in the biological and adoptive parents of people who were adopted as children; they found the following results:

	Prevalence of schizophrenia	
Adoptees	**Biological parents**	**Adoptive parents**
With schizophrenia	12	2
Without schizophrenia	6	4

[Data from S. S. Kety et al., 1978, in *The Nature of Schizophrenia: New Approaches to Research and Treatment*, L. C. Wynne, R. L. Cromwell, and S. Matthysse, Eds. New York: Wiley, pp. 25–37.]

What can you conclude from these results concerning the role of genetics in schizophrenia? Explain your reasoning.

36. Which conclusions are supported by **Figure 6.13**?

    a. Adoptive fathers of obese children have a higher BMI than adoptive fathers of thin children.

    b. Adoptive mothers of thin children have a lower BMI than adoptive mothers of obese children.

    c. Biological fathers of obese children have a higher BMI than adoptive fathers of thin children.

    d. Both a and b.

    e. Both a and c.

Section 6.4

37. What, if any, ethical issues might arise from the widespread use of noninvasive prenatal screening?

CHALLENGE QUESTIONS

Section 6.1

38. Many genetic studies, particularly those of recessive traits, have focused on small, isolated human populations, such as those on

islands. Suggest one or more advantages that isolated populations might have for the study of recessive traits.

Section 6.2

39. Draw a pedigree that represents an autosomal dominant trait, sex-limited to males, and that excludes the possibility that the trait is Y linked.

40.

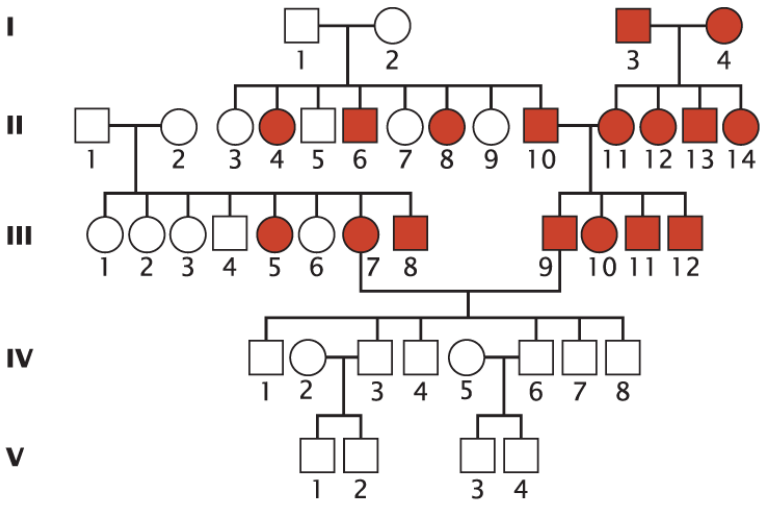
A. C. Stevenson and E. A. Cheeseman studied deafness in a family in Northern Ireland and recorded the following pedigree (A. C. Stevenson and E. A. Cheeseman. 1956. *Annals of Human Genetics* 20:177–231).

    a. If you consider only generations I through III, what is the most likely mode of inheritance for this type of deafness?

    b. Provide a possible explanation for the cross between III-7 and III-9 and the results for generations IV through V.

Section 6.3

41. Dizygotic twinning often runs in families, and its frequency varies among ethnic groups, whereas monozygotic twinning rarely runs in families, and its frequency is quite constant among ethnic groups. These observations have been interpreted as evidence of a genetic basis for variation in dizygotic twinning but little genetic basis for variation in monozygotic twinning. Can you suggest a possible reason for these differences in the genetic tendencies toward dizygotic and monozygotic twinning?



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[Data from A. C. Stevenson and E. A. Cheeseman. 1956. *Annals of Human Genetics* 20:177–231.]

Description

Generation 1 consists of 2 couples: one set of parents are both negative and the other set are both positive for the trait of deafness. The set of parents that are both positive for deafness have 3 positive females and 1 positive male offspring. The negative set of parents have 8 offsprings: 3 negative females, 1 negative male, 2 positive females, and 2 positive males. One of the positive males from this family mates with one of the positive females from the other family. They have 3 positive males and one positive female offspring. Another couple is shown in generation 2, They are both negative for the trait, but 3 of their 8 children are positive. One of the positive females from this family mates with one of the positive males from the other family in generation 3. They have 6 male offsprings, none of which express the trait for deafness. 2 of these males mate with negative partners, and they have 4 male offspring, none possessing the trait for deafness.

🗣️ ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 6.1

1. Pedigrees are used in the study of some nonhuman organisms. (a) List some nonhuman organisms for which pedigrees are often used. (b) What characteristics of these organisms make pedigree analysis practical and useful?

Section 6.2

2. How would the presence of incomplete penetrance (see Chapter 5) affect the interpretation of pedigrees? Do you think it would more strongly affect the interpretation of autosomal recessive or autosomal dominant traits? Explain your reasoning.

3. Explain why X-linked recessive traits are often more common in males, but X-linked dominant traits are often more common in females.

4. Why are there so few Y-linked traits in humans? (Hint: See Chapter 4.)

Section 6.3

5. The frequency of dizygotic twinning is higher in older women. Can you think of some possible evolutionary reasons for the

occurrence of more dizygotic twins in older women?

6. Trait X displays 98% concordance in both monozygotic and dizygotic twins. Do these concordances suggest that trait X is influenced by (a) genetic factors; (b) environmental factors; or (c) both genetic and environmental factors? Explain your reasoning.

7. Huntington disease (HD) is a genetic disorder that usually first appears in middle age and, over time, leads to involuntary movements, impaired speech, difficulty swallowing and speaking, and cognitive decline. There is no cure for HD, and affected individuals eventually die, usually after 10 to 15 years of progressively worsening symptoms. Huntington disease is inherited as an autosomal dominant disorder. Presymptomatic genetic testing for HD is available for people with a family history of the disease.

   Individuals who have a parent with HD have a 50% chance of inheriting the HD gene and eventually having HD. Some of these individuals want to know if they will eventually get the disease, and they undergo presymptomatic genetic testing. Others do not want to know, given that there is no cure or effective treatment for the disease. If you were at risk for HD, would you want to undergo genetic testing? What are some reasons for and against having a genetic test for HD?

8. Many genetic counselors will not provide presymptomatic genetic testing for Huntington disease to people younger than

age 18. Why are there concerns about offering this and many other genetic tests to minors? What types of presymptomatic genetic testing might be appropriate for minors?

9. Do you think direct-to-consumer tests that provide information about the risk of developing medical conditions (such as breast cancer or Alzheimer disease) should be made available to the public? List some arguments for and against providing this service.

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Draw a concept map relating the following terms in the introductory story about missing fingerprints: fingerprints, dermatoglyphic patterns, Francis Galton, adermatoglyphia, immigration delay disease, autosomal dominant inheritance, SNPs, *SMARCAD1*, abnormal splicing

Section 6.2

2. Create a concept map that illustrates the different modes of inheritance and at least two characteristics of each.

Section 6.3

3. Draw a concept map that outlines major concepts and terms associated with twin studies and adoption studies.

Section 6.4

4. Draw a concept map that outlines the major types of genetic testing.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 7

Linkage, Recombination, and Eukaryotic Gene Mapping

Benjamin Pierce.

Genes associated with variation in fear and aggression in dogs have been mapped to several specific chromosomal locations using genome-wide association studies. Pictured here is Molly, a dog who exhibits little fear and aggression.

Mapping Fear and Aggression in Dogs

Our Labrador retriever, Molly, is the most laid-back dog I've ever known. Molly doesn't understand the definition of a stranger and greets all visitors to our house with a vigorous wag of her tail. She is equally amiable toward other dogs. Confronted with an aggressive dog, she simply rolls over on her back. Nothing seems to make her anxious or fearful. She even loves going to the veterinarian, where she greets other dogs and the staff with great enthusiasm.

Not all dogs are so inclined, and indeed some breeds are consistently more aggressive and fearful than others. Fear and aggression have played important roles in dog evolution: compared to wild wolves, all domestic dogs have reduced aggression and fear toward humans, and early humans undoubtedly selected for tameness when they first domesticated dogs from their wolf ancestors some 30,000 years ago.

For many years, geneticists have been interested in the genetic underpinnings of behavioral traits like fear and aggression, not just in dogs but also in humans. This information could potentially be used to treat behavioral illnesses in humans that include fear, anxiety, and aggression. Unfortunately, studying the genetic basis of behavioral variation in humans is challenging because of the complexity of behavioral traits, long generation time, the inability to conduct controlled crosses, and small family size (see Chapter 6). Thus, geneticists have recently turned to dogs as a model for understanding how genes affect behavior.

In 2016, Isain Zapata and his colleagues published the results of a study that mapped genes affecting fear and aggression in dogs. To locate genes involved in these behaviors, they conducted a genome-wide association study, in which they looked for statistical associations between behavioral traits among dogs from 11 breeds and 175,000 genetic markers called single nucleotide polymorphisms (SNPs, pronounced “snips”). Scattered across the genome, SNPs are positions at which individuals differ in a single nucleotide. (The details of locating genes with genome-wide association studies are discussed later in this chapter). Genome-wide association studies in dogs are facilitated by the fact that dogs in different breeds are genetically distinct, while dogs within breeds are genetically similar.

What the researchers found was that fear in the dogs toward human strangers, as well as aggression toward both strange humans and unfamiliar dogs, was associated with genetic markers at two specific places in the genome: one spot on chromosome 18 and another on the X chromosome. Interestingly, aggression toward the dogs’ owners appeared to have a different genetic cause and was associated with different genes on chromosomes 15 and 34. The researchers were able to correlate these chromosomal locations to several known genes, some of which are expressed in those portions of the brain that are known to be associated with fear and aggression. These genes also showed signatures of strong past selection, suggesting that through artificial selection during dog

domestication, humans helped shape the variation found today at these genes.

To validate their findings, the researchers conducted another study on a different cohort of dogs, and results confirmed their earlier finding of associations of loci on chromosomes 18 and X with fear and aggression directed toward strangers and other dogs. Finally, the researchers used their genetic results to create a model that, based on genetic makeup, could predict fear and aggression in dogs, and tested this model in a third group of dog breeds. The model had a 58% success rate of predicting the level of fear and aggression observed in the dog breeds based on the particular genetic variants they possessed, versus a 7% random chance of success.

THINK-PAIR-SHARE

- Dogs are increasingly being used as a genetic model for the study of human traits and behaviors. Why are dogs a good genetic model organism for understanding human traits? Can you also think of some reasons why dogs are *not* a good genetic model organism? (Hint: See Chapter 1 for a discussion of model genetic organisms.)

This chapter explores the inheritance of genes and genetic markers located on the same chromosome. These linked genes do not strictly obey Mendel's principle of independent assortment; rather, they tend to be inherited together. This tendency requires a new approach to understanding their inheritance and predicting the

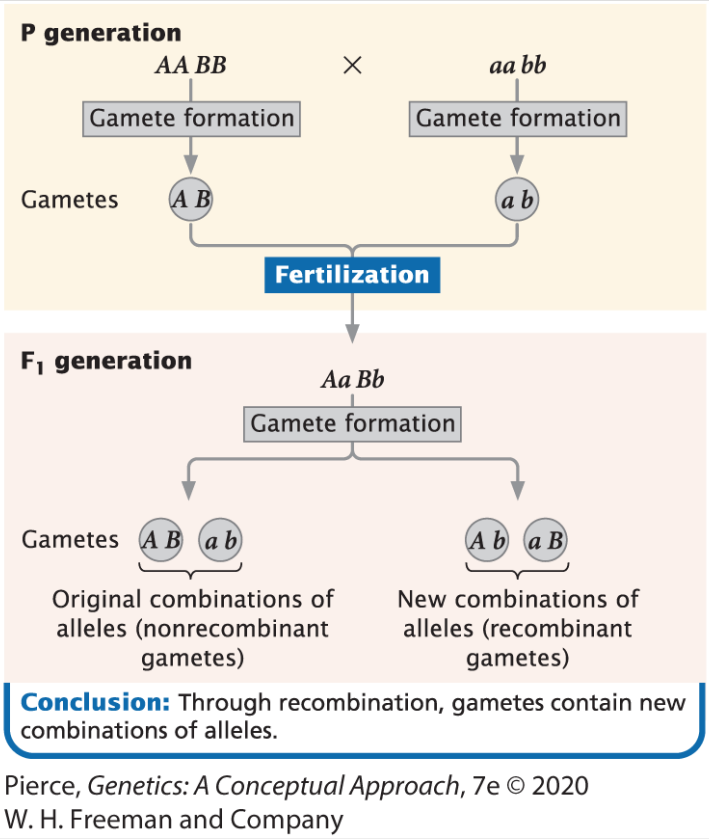
types of offspring that will be produced by a cross. A critical piece of information necessary for making these predictions is the arrangement of the genes on the chromosomes; thus, it will be necessary to think about the relation between genes and chromosomes. A key to understanding the inheritance of linked genes is to make the conceptual connection between the genotypes in a cross and the behavior of chromosomes in meiosis.

We begin our exploration of linkage by comparing the inheritance of two linked genes with the inheritance of two genes that assort independently. We then examine how recombination breaks up linked genes. Next, we use our knowledge of linkage and recombination for predicting the results of genetic crosses in which genes are linked as well as for mapping genes. Later in the chapter, we focus on physical methods of determining the chromosomal locations of genes. The final section examines variation in rates of recombination.

7.1 Linked Genes Do Not Assort Independently

Chapter 3 introduced Mendel's principles of segregation and independent assortment. Let's take a moment to review these two important concepts. The principle of segregation states that each diploid organism possesses two alleles at a locus that separate in meiosis, and that one allele passes into each gamete. The principle of independent assortment provides additional information about the process of segregation: it tells us that the two alleles at a locus separate independently of alleles at other loci.

The independent separation of alleles results in *recombination,* the sorting of alleles into new combinations. Consider a cross between individuals that are homozygous for two different pairs of alleles: *AA BB* × *aa bb*. The first parent, *AA BB*, produces gametes with the alleles *A B*, and the second parent, *aa bb*, produces gametes with the alleles *a b*, resulting in F_1 progeny with genotype *Aa Bb* (**Figure 7.1**). *Recombination* means that when one of the F_1 progeny reproduces, the combination of alleles in its gametes may differ from the combinations in the gametes of its parents. In other words, the F_1 may produce gametes with new allele combinations *A b* or *a B* in addition to parental gametes *A B* or *a b*.



7.1 Recombination is the sorting of alleles into new combinations.

Description

P generation at the top shows a cross between two parents with following genotypes: big A big A big B big B and small A small A small B small B. Their respective gametes formed are as follows: big A big B and small A small B. Fertilization occurs and F subscript 1 generation is obtained below. The F subscript 1 generation has offspring with the following genotype: big A small A big B small B. Gamete formation occurs and 2 pairs of gametes are formed: First pair: big A big B and small A small B. A text below reads, Original combinations of alleles (nonrecombinant gametes). The second pair: big A small B and small A big B. A caption below reads, New combinations of alleles (recombinant gametes).

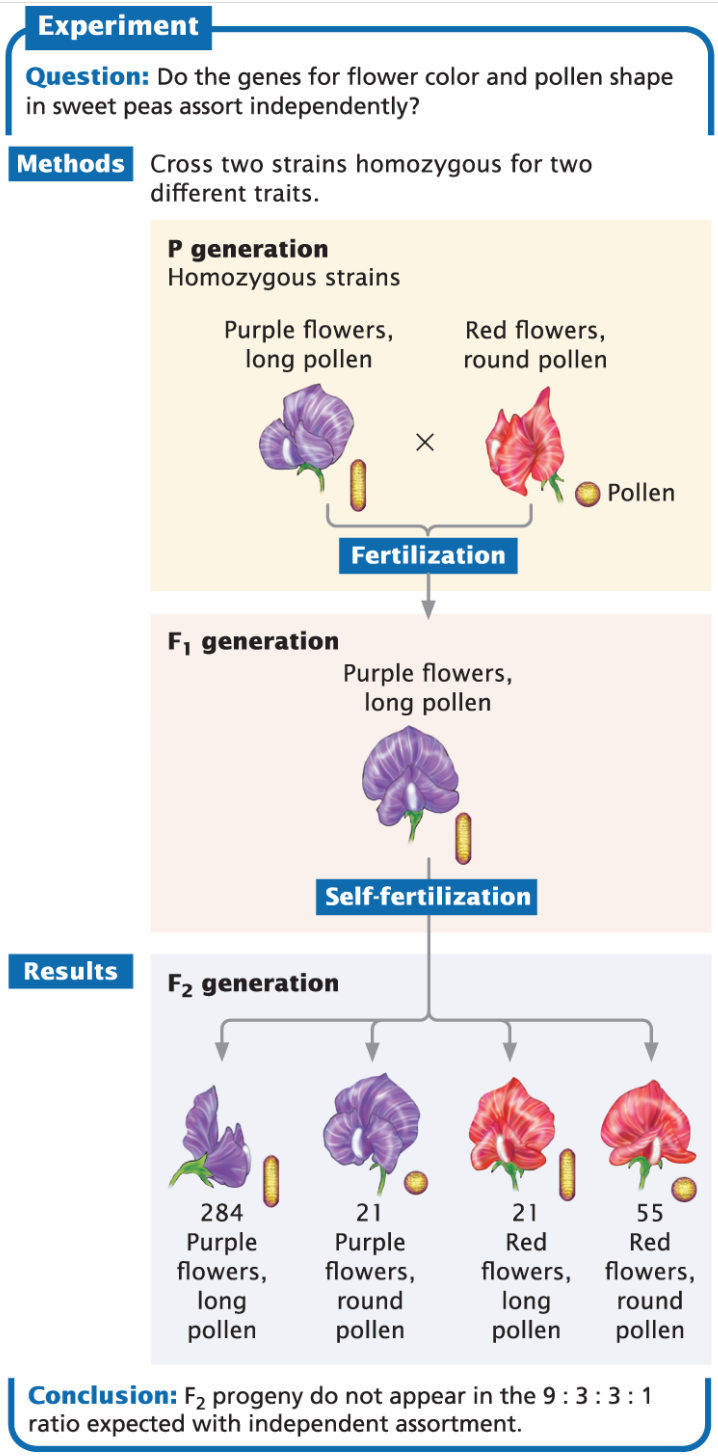
Mendel derived his principles of segregation and independent assortment by observing the progeny of genetic crosses, but he had no idea what biological processes produced these phenomena. In

1903, Walter Sutton proposed a biological basis for Mendel's principles, called the chromosome theory of heredity, which holds that genes are found on chromosomes (see Chapter 3). Let's restate Mendel's two principles in relation to the chromosome theory of heredity. The principle of segregation states that a diploid organism possesses two alleles for a characteristic, each of which is located at the same position, or locus, on one of the two homologous chromosomes. These chromosomes segregate in meiosis, and each gamete receives one homolog. The principle of independent assortment states that in meiosis, each pair of homologous chromosomes assorts independently of other homologous pairs. With this new perspective, it is easy to see that because the number of chromosomes in most organisms is limited, there are certain to be more genes than chromosomes, so some genes must be present on the same chromosome and should not assort independently. Genes located close together on the same chromosome are called **linked genes** and belong to the same **linkage group**. Linked genes travel together in meiosis, eventually arriving at the same destination (the same gamete), and are not expected to assort independently. All of the characteristics examined by Mendel in peas did display independent assortment, and the first genetic characteristics studied in other organisms also seemed to assort independently. How could genes be carried on a limited number of chromosomes and yet assort independently?

This apparent inconsistency between the principle of independent assortment and the chromosome theory of heredity soon

disappeared as biologists began finding genetic characteristics that did not assort independently. One of the first cases was reported in sweet peas by William Bateson, Edith Rebecca Saunders, and Reginald C. Punnett in 1905. They crossed a homozygous strain of peas that had purple flowers and long pollen grains with a homozygous strain that had red flowers and round pollen grains. All the F_1 had purple flowers and long pollen grains, indicating that purple was dominant over red and long was dominant over round. When they intercrossed the F_1, however, the resulting F_2 progeny did not appear in the 9 : 3 : 3 : 1 ratio expected with independent assortment (Figure 7.2). An excess of F_2 plants had purple flowers and long pollen or red flowers and round pollen (the parental phenotypes). Although Bateson, Saunders, and Punnett were unable to explain these results, we now know that the two loci that they examined lie close together on the same chromosome and therefore do not assort independently.

THINK-PAIR-SHARE Question 1



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7.2 Nonindependent assortment of flower color and pollen shape in sweet peas.

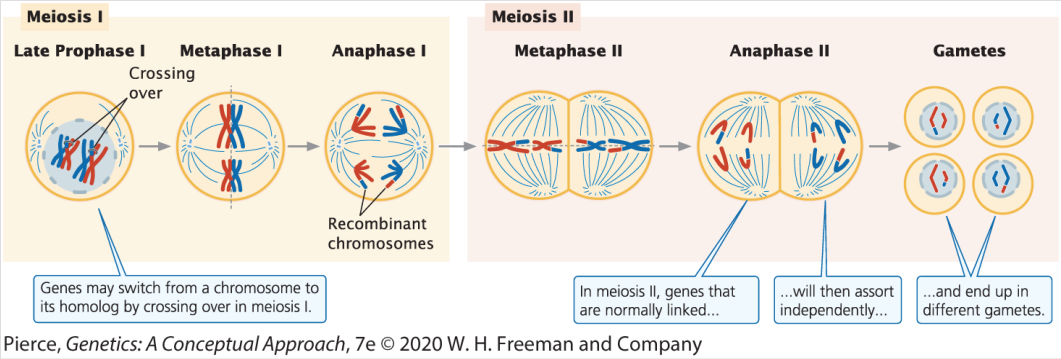
Description

The figure begins with the question as follows: Do the genes for flower color and pollen shape in sweet peas assort independently? The methods below involve the following text: Cross two strains homozygous for two different traits. P generation below shows a purple flower with long pollen crossed with a red flower with round pollen. Fertilization occurs and F subscript 1 generation is obtained below where all are purple flowers with long pollen. Self-fertilization occurs and results show F subscript 2 generation below. The results include F subscript 2 generation that has 284 purple flowers with long pollen, 21 purple flowers with round pollen, 21 red flowers with long pollen, and 55 red flowers with round pollen. A caption below reads, Conclusion: F subscript 2 progeny do not appear in the 9 is to 3 is to 3 is to 1

ratio expected with independent assortment

7.2 Linked Genes Segregate Together While Crossing Over Produces Recombination Between Them

Genes that are close together on the same chromosome usually segregate as a unit and are therefore inherited together. However, genes occasionally switch from one homologous chromosome to the other through the process of crossing over (see Chapter 2), as illustrated in **Figure 7.3**. Crossing over results in recombination: it breaks up the associations of genes that are close together on the same chromosome. Linkage and crossing over can be seen as processes that have opposite effects: linkage keeps particular genes together, and crossing over mixes them up, producing new combinations of genes. In Chapter 5, we considered a number of exceptions and extensions to Mendel's principles of heredity. The concept of linked genes adds a further complication to interpretations of the results of genetic crosses. However, with an understanding of how linkage affects heredity, we can analyze crosses for linked genes and successfully predict the types of progeny that will be produced.



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7.3 Crossing over takes place in meiosis and is responsible for recombination.

Description

Meiosis 1 on the left has late prophase 1, metaphase 1, and anaphase 1 stages. In the cells, there are 2 sets of homologous chromosomes where each one has a red and a blue counterpart within the nuclear envelope. A caption reads, Genes may switch from a chromosome to its homolog by crossing over in meiosis 1. During metaphase 1, spindle fibers are formed from one cell pole to another and new combination of a chromosome is represented by the hybrid color pattern in the recombinant chromosome. These chromosomes now align at the center of the cell. During anaphase 1, the recombined chromosomes are being pulled apart. Meiosis 2 on the right shows metaphase 2, anaphase 2, and the resulting gametes. During metaphase 2, the cell has two compartments and the spindle fibers are now drawn vertically in each of the compartments. The chromosomes are now aligned horizontally at the center of the cell. During anaphase 2, each sister chromatid is pulled towards the cell poles. A caption reads, In meiosis 2, genes that are normally linked will then assort independently and end up in different gametes. Four gametes are formed, and a portion of one of the 2 chromosomes is recombined. The chromosomes are in nuclear envelope in each of the gametes formed.

Notation for Crosses with Linkage

In analyzing crosses with linked genes, we must know not only the genotypes of the individuals crossed but also the arrangement of the genes on the chromosomes. To keep track of this arrangement, we introduce a new system of notation for presenting crosses with linked genes. Consider a cross between an individual homozygous for dominant alleles at two linked loci and another individual homozygous for recessive alleles at those loci (*AA BB* × *aa bb*). For linked genes, it's necessary to write out the specific alleles as they are arranged on each of the homologous chromosomes:

$$\frac{A \quad B}{A \quad B} \times \frac{a \quad b}{a \quad b}$$

Description
The homologous chromosomes are indicated by a double line. The first pair has big A gene on the far left and the big B gene on the far right for both the chromosomes. The second pair has small A gene on the left end and small B on the right end for both the chromosomes.

In this notation, each horizontal line represents one of the two homologous chromosomes. Because they inherit one chromosome from each parent, the F_1 progeny will have the following genotype:

$$\frac{A \quad B}{a \quad b}$$

Description
The drawing shows genes big A on the left and big B on the right, above the horizontal straight line representing the top chromosome. A parallel line below represents bottom homologous chromosome. Below this line, small A is on the left and small B is on the right.

Here, the importance of designating the alleles on each chromosome is clear. One chromosome has the two dominant alleles A and B, whereas the homologous chromosome has the two recessive alleles a and b. The notation can be simplified by drawing only a single line, with the understanding that genes located on the same side of the line lie on the same chromosome:

$$\frac{A \quad B}{a \quad b}$$

Description
A single line represents a pair of homologous chromosomes. Above the line, big A is on the left and big B is on the right. Below the line, small A is on the left and small B is on the right.

This notation can be simplified further by separating the alleles on each chromosome with a slash: AB/ab.

Remember that the two alleles at a locus are always located on different homologous chromosomes and therefore must lie on opposite sides of the line. Consequently, we would *never* write the genotypes as

$$\frac{A \quad a}{B \quad b}$$

Description
A single line represents a pair of homologous chromosomes. Above the line, big A is on the left and small A is on the right. Below the line, big B is on the left and small B is on the right.

because the alleles A and a can *never* be on the same chromosome. It is also important to keep the genes in the same order on both sides of the line; thus, we would *never* write

$$\frac{A \quad B}{b \quad a}$$

Description
A single line represents a pair of homologous chromosomes. Above the line, big A is on the left and big B is on the right. Below the line, small B is on the left and small A is on the right.

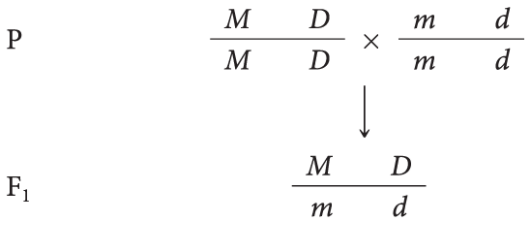
because it would imply that alleles A and b are allelic (at the same locus).

Complete Linkage Compared with Independent Assortment

In this section, we first consider what happens to genes that exhibit complete linkage, meaning that they are located very close together on the same chromosome and do not exhibit crossing over. Genes are rarely completely linked, but by assuming that no crossing over takes place, we can see the effect of linkage more clearly. We then consider what happens when genes assort independently. Finally, we consider the results obtained if the genes are linked but exhibit some crossing over.

A testcross reveals the effects of linkage. For example, if a heterozygous individual is test-crossed with a homozygous recessive individual (*Aa Bb* × *aa bb*), the alleles that are present in the gametes contributed by the heterozygous parent will be expressed in the phenotype of the offspring because the homozygous parent cannot contribute dominant alleles that might mask them. Consequently, traits that appear in the progeny reveal which alleles were transmitted by the heterozygous parent.

Consider a pair of linked genes in tomato plants. One of the genes affects the type of leaf: an allele for mottled leaves (*m*) is recessive to an allele that produces normal leaves (*M*). Nearby on the same chromosome, another gene determines the height of the plant: an allele for dwarf (*d*) is recessive to an allele for tall (*D*). Testing for linkage can be done with a testcross, which requires a plant that is heterozygous for both characteristics. A geneticist might produce this heterozygous plant by crossing a variety of tomato that is homozygous for normal leaves and tall height with a variety that is homozygous for mottled leaves and dwarf height:



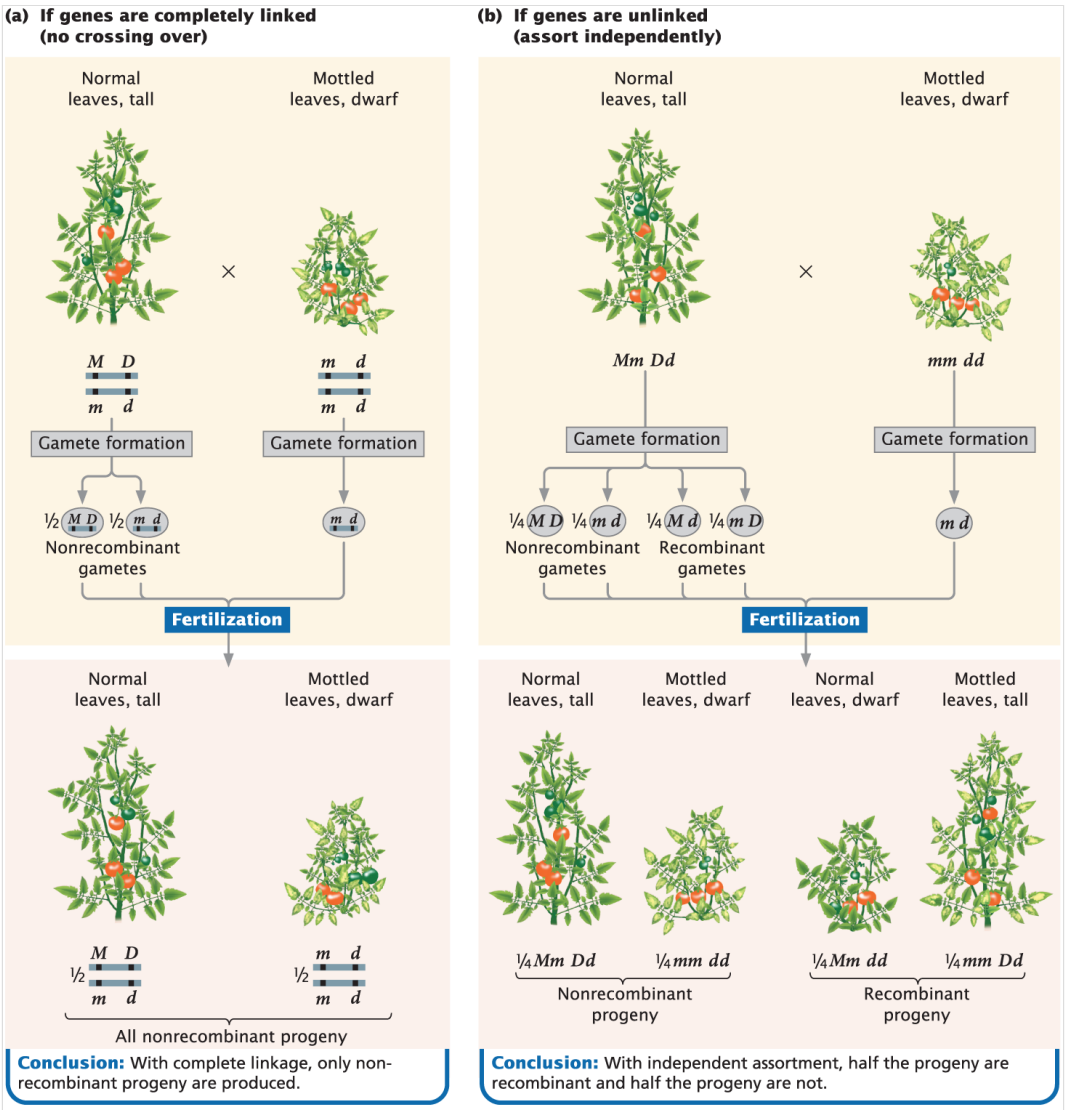
Description
In the P generation at the top, one parent has big M on the left and big D on the right, both above and below the line. The other parent has small M on the left and small D on the right,

both above and below the line. Crossing takes place and F subscript 1 generation is obtained below. This generation shows progeny with big M on the left and big D on the right, on the top of the line and small M on the left and small D on the right, below the line.

The geneticist would then use this F_1 heterozygote in a testcross, crossing it with a plant that is homozygous for mottled leaves and dwarf height:

$$\frac{M \quad D}{M \quad D} \times \frac{m \quad d}{m \quad d}$$

The results of this testcross are diagrammed in **Figure 7.4a**. The heterozygote produces two types of gametes: some with the $\underline{M \quad D}$ chromosome and others with the $\underline{m \quad d}$ chromosome. Because no crossing over takes place, these gametes are the only types produced by the heterozygote. Notice that the gametes produced by the heterozygote contain only combinations of alleles that were present in its parents (the P generation): either the allele for normal leaves together with the allele for tall height (*M* and *D*) or the allele for mottled leaves together with the allele for dwarf height (*m* and *d*). Gametes that contain only original combinations of alleles that were present in the parents are **nonrecombinant gametes**, or *parental gametes*.



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7.4 A testcross reveals the effects of linkage. Results of a testcross for two loci in tomatoes that determine leaf type and plant height.

Description

Part a on the left shows the results when genes are completely linked (no crossing over). The P generation has a tall tomato plant with normal leaves crossed with a dwarf tomato plant with mottled leaves. The tall plant has genes big M on the left and big D on the right, above the line and small M on the left and small D on the right, below the line. The dwarf plant has small M on the left and small D on the right, both above and below the line. Gamete formation occurs. For tall plant, nonrecombinant gametes are formed where one-half gametes have big M big D and one-half gametes have small M small D. For dwarf

plant, gametes have small M small D. Fertilization occurs and in F subscript 1 generation below, one-half progeny are tall with normal leaves with genotype big M big D small M small D while one-half progeny are dwarf with mottled leaves with genotype small M small D small M small D. All the progenies are nonrecombinant. A caption reads, Conclusion: With complete linkage, only nonrecombinant progeny are produced.

Part b on the right shows the results if genes are unlinked (assort independently). It shows a cross between a tall plant with normal leaves and a dwarf plant with mottled leaves. The tall plant has the genotype big M small M big D small D. The dwarf plant has the genotype small M small M small D small D. Gamete formation occurs. The tall plant has the following gametes: one-fourth big M big D and one-fourth small M small D that are nonrecombinant; one-fourth big M small D and one-fourth small M big D that are recombinant gametes. Dwarf plant has following gametes: small M small D. Fertilization occurs and the following progenies are produced in F subscript 1 generation:

Nonrecombinant progeny: one-fourth big M small M big D small D (tall plants with normal leaves) and one-fourth small M small M small D small D (dwarf plants with mottled leaves).

Recombinant progeny: one-fourth big M small M small D small D (dwarf plants with normal leaves) and one-fourth small M small M big D small D (tall plants with mottled leaves). A caption below reads, Conclusion: With independent assortment, half the progeny are recombinant and half the progeny are not.

The homozygous parent in the testcross produces only one type of gamete, which contains chromosome $\underline{m \quad d}$. When its gametes pair with the two types of gametes generated by the heterozygous parent (see Figure 7.4a), two types of progeny result: half have normal leaves and are tall:

$$\frac{M \quad D}{m \quad d}$$

and half have mottled leaves and are dwarf:

$$\frac{m \quad d}{m \quad d}$$

These progeny display the original combinations of traits present in the P generation and are **nonrecombinant progeny**, or *parental progeny*. No new combinations of the two traits, such as normal leaves with dwarf height or mottled leaves with tall height, appear in the offspring because the genes affecting the two traits are completely linked and are inherited together. New combinations of traits could arise only if the physical connection between *M* and *D* or between *m* and *d* were broken.

These results are distinctly different from the results that are expected when genes assort independently (**Figure 7.4b**). If the *M* and *D* loci assorted independently, the heterozygous plant (*Mm Dd*) would produce four types of gametes: two nonrecombinant gametes containing the original combinations of alleles (*M D* and *m d*) and two gametes containing new combinations of alleles (*M d* and *m D*). Gametes with new combinations of alleles are called **recombinant gametes**. With independent assortment, nonrecombinant and recombinant gametes are produced in equal proportions. These four types of gametes join with the single type of gamete produced by the homozygous parent of the testcross to produce four kinds of progeny in equal proportions (see Figure 7.4b). The progeny with new combinations of traits formed from recombinant gametes are termed **recombinant progeny**.

CONCEPTS

A testcross in which one of the individuals is heterozygous for two completely linked genes yields two types of progeny, each type displaying one of the original combinations of traits present in the P generation. In contrast, independent assortment produces four types of progeny in a 1: 1: 1: 1 ratio—two types of recombinant progeny and two types of nonrecombinant progeny in equal proportions.

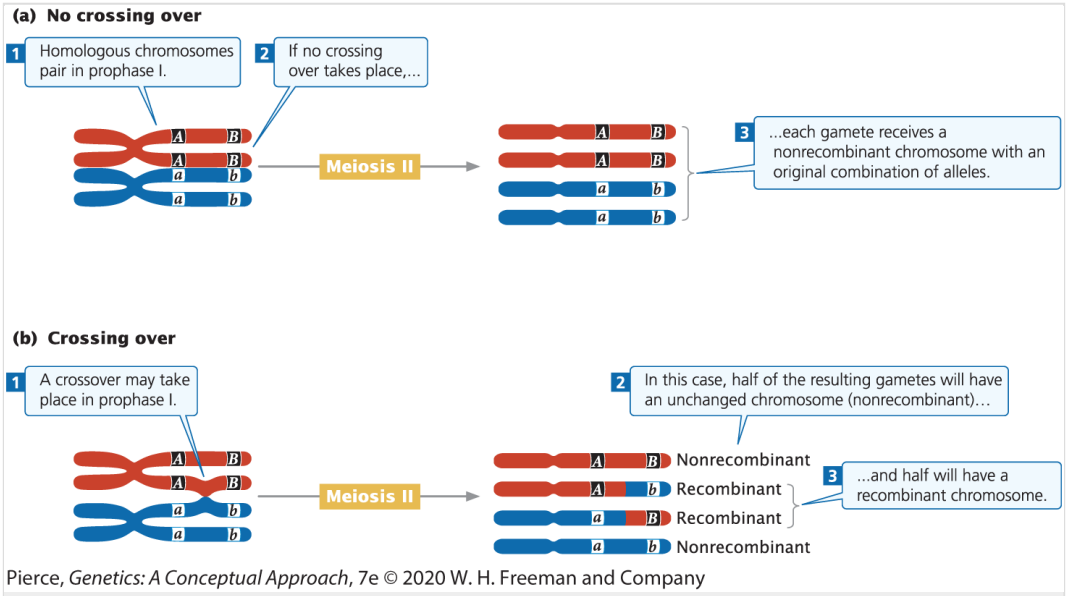
Crossing Over Between Linked Genes

Usually, there is some crossing over between genes that lie on the same chromosome, which produces new combinations of traits. Genes that exhibit crossing over are said to be incompletely linked. Let's see how incomplete linkage affects the results of a cross.

THEORY

The effect of crossing over on the inheritance of two linked genes is shown in **Figure 7.5**. Crossing over, which takes place in prophase I of meiosis, is the exchange of genetic material between nonsister chromatids (see Figures 2.16 and 2.18). After a single crossover has taken place, the two chromatids that did not participate in crossing over are unchanged; gametes that receive these chromatids are nonrecombinants. The other two chromatids, which did participate in crossing over, now contain new combinations of alleles; gametes

that receive these chromatids are recombinants. For each meiosis in which a single crossover takes place, two nonrecombinant gametes and two recombinant gametes are produced. This result is the same as that produced by independent assortment (see Figure 7.4b), so if crossing over between two loci takes place in every meiosis, it is impossible to determine whether the genes are on the same chromosome and crossing over took place or whether the genes are on different chromosomes.



7.5 A single crossover produces half nonrecombinant gametes and half recombinant gametes.

Description

Part a at the top shows the arrangement of gametes when no crossing over occurs. Two X-shaped chromosomes are drawn one below the other where one is red and the other is blue. The chromosome above has big A on the left and big B on the right of its long arm of top chromatid and big A on the left and big B on the right of the long arm of below sister chromatid. The chromosome below has small A on the left and small B on the right of the long arm of the top chromatid and small A on the left and small B on the right of the long

arm of the below chromatid. A caption reads, Homologous chromosomes pair in prophase 1. Another caption reads, If no crossing over takes place each gamete receives a nonrecombinant chromosome with an original combination of alleles. Meiosis 2 occurs and 4 gametes are formed where 2 gametes are red and have big A on the left and big B on the right of their respective long arm, while 2 gametes are blue and have small A on the left and small B on the right of their respective long arm.

Part b below shows the arrangement of gametes when crossing over occurs. Two X-shaped chromosomes are drawn one above the other where one is red and the other is blue. At one point on the long arms of one sister chromatid of one chromosome and one sister chromatid of the other chromosome, they are joined. The red chromosome has big A on the left and big B on the right of the long arm of the top chromatid and big A on the left and big B on the right of the below sister chromatid. The blue chromosome below has small A on left and small B on the right of the long arm of top chromatid and small A on the left and small B on the right of the below chromatid. A caption reads, A crossover may take place in prophase 1. Meiosis 2 occurs and 4 gametes are formed. The first gamete is red and has big A on the left and big B on the right of its long arm (non-recombinant). The second gamete is red with big A on the left and small B (blue part) on the right of the long arm (recombinant). The third gamete is blue and has small A on the left and big B (red part) on the right of the long arm (recombinant). The fourth gamete is blue and has small A on left and small B on the right of its long arm (non-recombinant). A caption reads, In this case, half of the resulting gametes will have an unchanged chromosome (nonrecombinant) and half will have a recombinant chromosome.

For closely linked genes, however, crossing over does not take place in every meiosis. In meioses in which there is no crossing over, only nonrecombinant gametes are produced. In meioses in which there is a single crossover, half the gametes are recombinants and half are nonrecombinants (because a single crossover affects only two of the four chromatids). Because each crossover leads to half recombinant gametes and half nonrecombinant gametes, the total percentage of recombinant gametes is always half the percentage of meioses in

which crossing over takes place. Even if crossing over between two genes takes place in every meiosis, only 50% of the resulting gametes are recombinants. Thus, the frequency of recombinant gametes is always half the frequency of crossing over, and the maximum proportion of recombinant gametes is 50%.

CONCEPTS

Linkage between genes causes them to be inherited together and reduces recombination; crossing over breaks up the associations of such genes. In a testcross for two linked genes, each crossover produces two recombinant gametes and two nonrecombinants. The frequency of recombinant gametes is half the frequency of crossing over, and the maximum frequency of recombinant gametes is 50%.

✔ CONCEPT CHECK 1

For single crossovers, the frequency of recombinant gametes is half the frequency of crossing over because

a. a testcross between a homozygote and a heterozygote produces $^1/_2$ heterozygous and $^1/_2$ homozygous progeny.
b. the frequency of recombination is always 50%.
c. each crossover takes place between only two of the four chromatids of a homologous pair.
d. crossovers take place in about 50% of meioses.

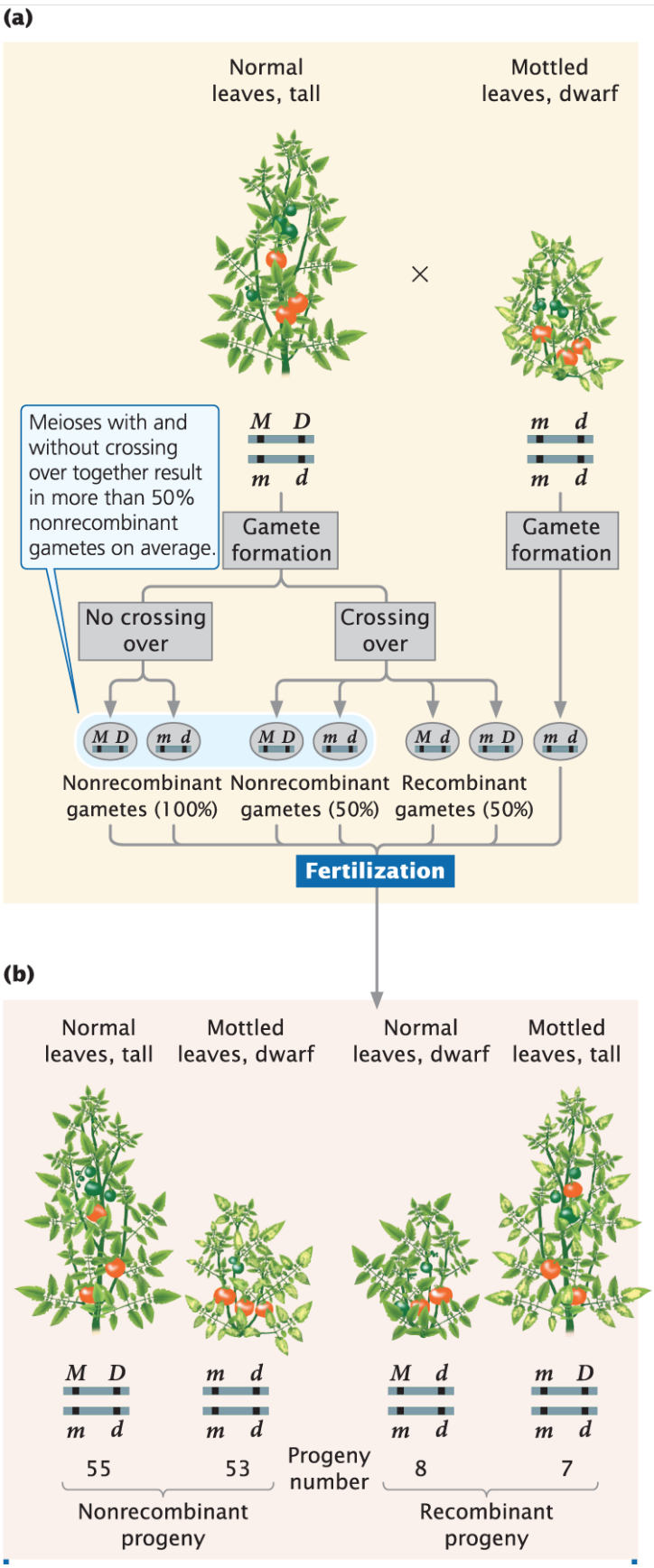
APPLICATION

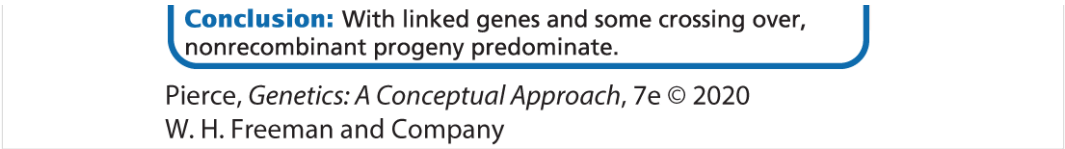
Let's apply what we have learned about linkage and recombination to a cross between tomato plants that differ in the genes that encode

leaf type and plant height. Assume now that these genes are linked and that some crossing over takes place between them. Suppose a geneticist carried out the testcross described earlier:

$$\frac{M \quad D}{m \quad d} \times \frac{m \quad d}{m \quad d}$$

When crossing over takes place between the genes for leaf type and height, two of the four gametes produced are recombinants. When there is no crossing over, all four resulting gametes are nonrecombinants. Because each crossover produces half recombinant gametes and half nonrecombinant gametes, the majority of gametes produced by the heterozygous parent will be nonrecombinants (**Figure 7.6a**). These gametes then unite with gametes produced by the homozygous recessive parent, which contain only the recessive alleles, resulting in mostly nonrecombinant progeny and a few recombinant progeny (**Figure 7.6b**). In this testcross, we see that 55 of the progeny have normal leaves and are tall and that 53 have mottled leaves and are dwarf. These plants are the nonrecombinant progeny, containing the original combinations of traits that were present in the parents. Of the 123 progeny, 15 have new combinations of traits that were not seen in the parents: 8 have normal leaves and are dwarf, and 7 have mottled leaves and are tall. These plants are the recombinant progeny.


(a)
Normal leaves, tall
Mottled leaves, dwarf
×
Meioses with and without crossing over together result in more than 50% nonrecombinant gametes on average.
M D
m d
Gamete formation
m d
m d
Gamete formation
No crossing over
Crossing over
M D
m d
M D
m d
M d
m D
m d
Nonrecombinant gametes (100%)
Nonrecombinant gametes (50%)
Recombinant gametes (50%)
Fertilization
(b)
Normal leaves, tall
Mottled leaves, dwarf
Normal leaves, dwarf
Mottled leaves, tall
M D
m d
m d
m d
M d
m d
m D
m d
55
53
Progeny number
8
7
Nonrecombinant progeny
Recombinant progeny



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7.6 Crossing over between linked genes produces nonrecombinant and recombinant offspring. In this testcross, genes are linked, and there is some crossing over.

Description

Part a at the top shows the P generation. A tall tomato plant with normal leaves with genotype big M big D small M small D is crossed with a dwarf tomato plant with mottled leaves with genotype small M small D small M small D. Gamete formation occurs. The tall plant has the following gametes: When no crossing over occurs: 1 gamete has big M big D and 1 gamete has small M small D (these are nonrecombinant gametes 100 percent). When crossing over occurs: of the two left gametes, 1 gamete has big M big D and 1 gamete has small M small D (these are nonrecombinant gametes 50 percent). A callout refers to the non-crossing over gametes and the left two crossing over gametes and reads as follows, Meioses with and without crossing over together result in more than 50 percent nonrecombinant gametes on average. Of the two right gametes, 1 gamete has big M small D and 1 gamete has small M big D (these are recombinant gametes 50 percent). The dwarf plant has the following gametes: small M small D. Fertilization occurs and leads to part b below where F subscript 1 generation is obtained. It shows the following progeny:

Nonrecombinant progeny: 55 tall plants with normal leaves with genotype big M big D small M small D and 53 dwarf plants with mottled leaves with genotype small M small D small M small D.

Recombinant progeny: 8 dwarf plants with normal leaves with genotype big M small D small M small D and 7 tall plants with mottled leaves with genotype small M big D small M small D.

A caption below reads, Conclusion: With linked genes and some crossing over, nonrecombinant progeny predominate.

The results of a cross such as the one illustrated in Figure 7.6 reveal several things. A testcross for two independently assorting genes is expected to produce a 1 : 1 : 1 : 1 phenotypic ratio in the progeny. The progeny of this cross clearly do not exhibit such a ratio, so we might suspect that the genes are not assorting independently. When linked genes undergo some crossing over, the result is mostly nonrecombinant progeny and a few recombinant progeny. This result is what we observe among the progeny of the testcross illustrated in Figure 7.6, so we conclude that the two genes show evidence of linkage with some crossing over.

Calculating Recombination Frequency

The percentage of recombinant progeny produced in a cross is called the **recombination frequency** (or *rate of recombination*), which is calculated as follows:

$$\text{recombination frequency} = \frac{\text{number of recombinant progeny}}{\text{total number of progeny}} \times 100\%$$

In the testcross shown in Figure 7.6, 15 progeny exhibit new combinations of traits, so the recombination frequency is

$$\frac{8 + 7}{55 + 53 + 8 + 7} \times 100\% = \frac{15}{123} \times 100\% = 12.2\%$$

Thus, 12.2% of the progeny exhibit new combinations of traits resulting from crossing over. The recombination frequency can also

be expressed as a decimal fraction (0.122). **› TRY PROBLEM 15**

THINK-PAIR-SHARE Question 2

Coupling and Repulsion

In crosses for linked genes, the arrangement of alleles on the homologous chromosomes is critical in determining the outcome of the cross. For example, consider the inheritance of two genes in the Australian blowfly, *Lucilia cuprina*. In this species, one locus determines the color of the thorax: a purple thorax (p) is recessive to the normal green thorax (p^+). A second locus determines the color of the puparium: a black puparium (b) is recessive to the normal brown puparium (b^+). The loci for thorax color and puparium color are located close together on the same chromosome. Suppose that we test-cross a fly that is heterozygous at both loci with a fly that is homozygous recessive at both. Because these genes are linked, there are two possible arrangements on the chromosomes of the heterozygous fly. The dominant alleles for green thorax (p^+) and brown puparium (b^+) might reside on one chromosome of the homologous pair, and the recessive alleles for purple thorax (p) and black puparium (b) might reside on the other homologous chromosome:

$$\frac{p^+ \quad b^+}{p \quad b}$$

Description
The chromosomes are represented by a straight, horizontal line. The alleles above the line are small P superscript positive small B superscript positive and alleles below the line are small P small B.

This arrangement, in which wild-type alleles are found on one chromosome and mutant alleles are found on the other chromosome, is referred to as the **coupling**, or **cis, configuration**.

Alternatively, one chromosome might carry the alleles for green thorax (p^+) and black puparium (b), and the other chromosome might carry the alleles for purple thorax (p) and brown puparium (b^+):

$$\frac{p^+ \quad b}{p \quad b^+}$$

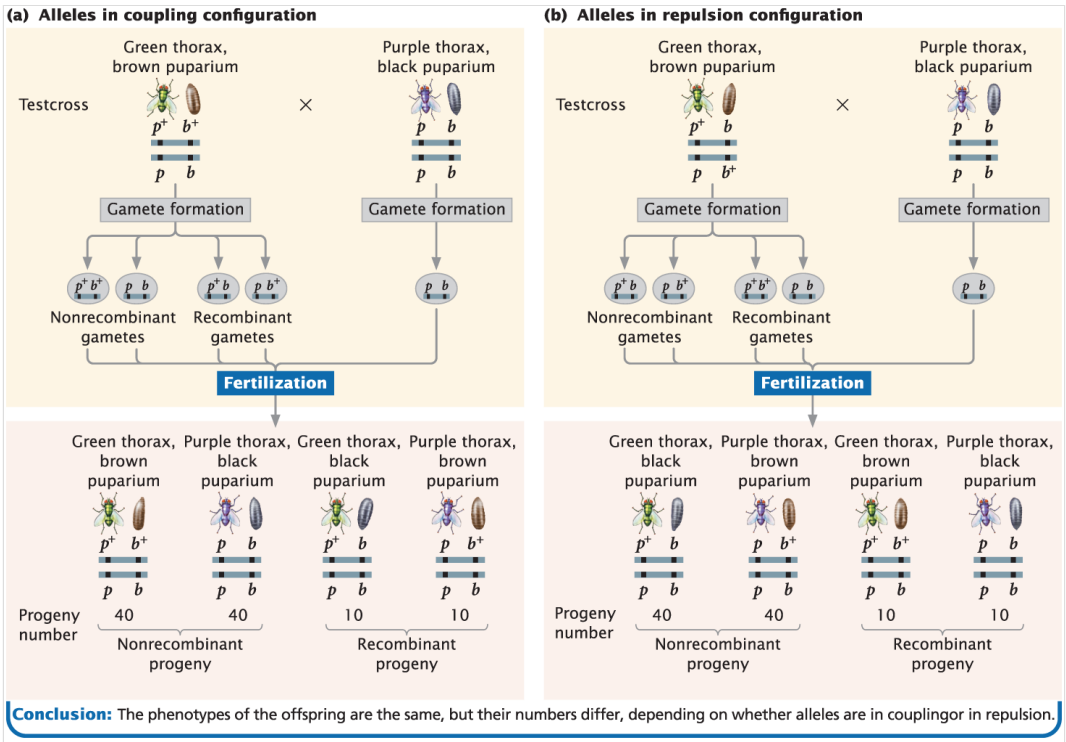
Description
The chromosomes are represented by a straight, horizontal line. The alleles above the line are small P superscript positive small B and alleles below the line are small P small B superscript positive.

This arrangement, in which each chromosome contains one wild-type and one mutant allele, is called the **repulsion**, or **trans, configuration**. Whether the alleles in the heterozygous parent are in

coupling or repulsion determines which phenotypes will be most common among the progeny of a testcross.

When the alleles of the heterozygous parent are in the coupling configuration, the most numerous progeny types are those with a green thorax and brown puparium and those with a purple thorax and black puparium (**Figure 7.7a**). However, when the alleles are in repulsion, the most numerous progeny types are those with a green thorax and black puparium and those with a purple thorax and brown puparium (**Figure 7.7b**). Notice that because the alleles are in repulsion, the phenotypes of the nonrecombinant progeny (green thorax with black puparium and purple thorax with brown puparium) are not the same as the phenotypes of the parents (green thorax with brown puparium and purple thorax with black puparium). Nonrecombinant progeny are those that resulted from a chromosome that did not undergo recombination and that need not have the same phenotype as the parents. Notice also that the genotypes of the parents in Figure 7.7a and b are the same ($p^+p\ b^+b \times pp\ bb$) and that the dramatic difference in the phenotypic ratios of the progeny in the two crosses results entirely from the configuration—coupling or repulsion—of the chromosomes. Knowledge of the arrangement of the alleles on the chromosomes is essential to accurately predicting the outcome of crosses in which genes are linked.

THINK-PAIR-SHARE Question 3



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7.7 The arrangement (coupling or repulsion) of linked genes on a chromosome affects the results of a testcross. Linked loci in the Australian blowfly (*Lucilia cuprina*) determine the color of the thorax and that of the puparium.

Description

Figure a on the left shows results when alleles are in coupling configuration. P generation shows a testcross between a fly with a green thorax and a brown puparium and a fly and a purple thorax and a black puparium. The genotype of the green fly is small P superscript positive small B superscript positive small P small B, while that of the purple fly is small P small B small P small B. Gamete formation occurs and the following gametes are formed: For the green fly, nonrecombinant gametes are small P superscript positive small B superscript positive and small P small B. Recombinant gametes are small P superscript positive small B and small P small B superscript positive. For the purple fly, gametes are small P small B. Fertilization occurs and the F subscript 1 generation is obtained below: Nonrecombinant progeny or flies: 40 green thorax brown puparium (small P superscript positive small B superscript positive small P small B) and 40 purple thorax black puparium (small P small B small P small B). Recombinant progeny or flies: 10 green thorax black puparium (small P superscript positive small B small P small B) and 10 purple thorax brown puparium (small P small B superscript positive small P small B).

Figure b on the right shows results when alleles are in repulsion configuration. In this test cross, P generation shows a green fly with brown puparium. Its genotype is small P superscript positive small B small P small B superscript positive. The green fly is crossed with a purple fly with black puparium, which has the genotype small P small B small P small B. Gamete formation occurs and following gametes are formed: For the green fly, nonrecombinant gametes are small P superscript positive small B and small P small B superscript positive. Recombinant gametes are small P superscript positive small B superscript positive and small P small B. For the purple fly, gametes formed are small P small B. Fertilization occurs and F subscript 1 generation is obtained below. Nonrecombinant progeny: 40 green thorax black puparium (small P superscript positive small B small P small B) and 40 purple thorax brown puparium (small P small B superscript positive small P small B). Recombinant progeny: 10 green thorax brown puparium (small P superscript positive small B superscript positive small P small B) and 10 purple thorax black puparium (small P small B small P small B). A caption below reads, Conclusion: The phenotypes of the offspring are the same, but their numbers differ, depending on whether alleles are in coupling or in repulsion.

CONCEPTS

The arrangement of linked alleles on the chromosomes is critical for determining the outcome of a cross. When two wild-type alleles are on one homologous chromosome and two mutant alleles are on the other, the alleles are in the coupling configuration; when each chromosome contains one wild-type allele and one mutant allele, the alleles are in repulsion.

✔ CONCEPT CHECK 2

The following testcross produces the progeny shown: *Aa Bb* × *aa bb* → 10 *Aa Bb*, 40 *Aa bb*, 40 *aa Bb*, 10 *aa bb.* Were the *A* and *B* alleles in the *Aa Bb* parent in coupling or in repulsion?

CONNECTING CONCEPTS CONNECT

Relating Independent Assortment, Linkage, and Crossing Over

We have now considered three situations for genes at different loci. First, the genes may be located on different chromosomes; in this case, they exhibit independent assortment and combine randomly when gametes are formed. An individual heterozygous at two loci (*Aa Bb*) produces four types of gametes (*A B*, *a b*, *A b*, and *a B*) in equal proportions: two types of nonrecombinants and two types of recombinants. In a testcross, these gametes will result in four types of progeny in equal proportions (**Table 7.1**).

TABLE 7.1 Results of a testcross (*Aa Bb* × *aa bb*) with complete linkage, independent assortment, and linkage with some crossing over

Situation	Progeny of Testcross	
Independent assortment	*Aa Bb* (nonrecombinant)	25%
	aa bb (nonrecombinant)	25%
	Aa bb (recombinant)	25%
	aa Bb (recombinant)	25%
Complete linkage (genes in coupling)	*Aa Bb* (nonrecombinant)	50%
	aa bb (nonrecombinant)	50%
Linkage with some crossing over	$Aa\ Bb$ (nonrecombinant) $aa\ bb$ (nonrecombinant)	More than 50%
(genes in coupling)	$Aa\ bb$ (recombinant) $aa\ Bb$ (recombinant)	Less than 50%

Second, the genes may be completely linked—meaning that they are on the same chromosome and lie so close together that crossing over between them is rare. In this case, the genes do not recombine. An individual heterozygous for two completely linked genes in the coupling configuration

$$\frac{A \quad B}{a \quad b}$$

Description

A single line represents a pair of homologous chromosomes. Above the line, big A is on the left and big B is on the right. Below the line, small A is on the left and small B is on the right.

produces only nonrecombinant gametes containing alleles *A B* or *a b*; the alleles do not assort into new combinations such as *A b* or *a B*. In a testcross, completely linked genes produce only two types of progeny, both nonrecombinants, in equal proportions (see Table 7.1).

The third situation, incomplete linkage, is intermediate between the two extremes of independent assortment and complete linkage. Here, the genes are physically linked on the same chromosome, which prevents independent assortment. However, occasional crossovers break up the linkage and allow the genes to recombine. With incomplete linkage, an individual heterozygous at two loci produces four types of gametes—two types of recombinants and two types of nonrecombinants—but the nonrecombinants are produced more frequently than are the recombinants because crossing over between these loci does not take place in every meiosis. In the testcross, these gametes result in four types of progeny, with the nonrecombinants more frequent than the recombinants (see Table 7.1).

Earlier in the chapter, the term *recombination* was defined as the sorting of alleles into new combinations. We've now considered two types of recombination that differ in their mechanisms. Interchromosomal recombination takes place between genes located on *different* chromosomes. It arises from independent assortment—the random segregation of chromosomes in anaphase I of meiosis—and is the kind of recombination that Mendel discovered while studying dihybrid crosses. A second type of recombination, intrachromosomal recombination, takes place between genes located on the *same* chromosome. This recombination arises from crossing over—the exchange of genetic material in prophase I of meiosis. Both recombination mechanisms produce new allele combinations in the gametes, so they cannot be distinguished by examining the types of gametes produced. Nevertheless, they can often be distinguished by the *frequencies* of types of gametes: interchromosomal recombination produces 50% nonrecombinant gametes and 50% recombinant gametes, whereas intrachromosomal recombination frequently produces more than 50% nonrecombinant gametes and less than 50% recombinant gametes. However, when the genes are very far apart on the same chromosome, crossing over takes place in every meiotic division, leading to 50% recombinant gametes and 50% nonrecombinant gametes. This result is the same as in independent assortment of genes located on different chromosomes (interchromosomal recombination). Thus, intrachromosomal recombination of

genes that lie far apart on the same chromosome and interchromosomal recombination are phenotypically indistinguishable.

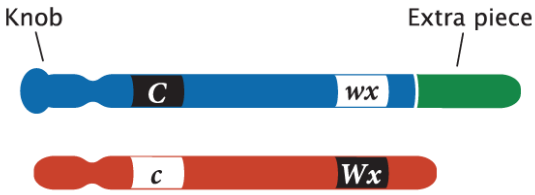
Evidence for the Physical Basis of Recombination

Walter Sutton's chromosome theory of inheritance, which stated that genes are physically located on chromosomes (see Chapter 3), was supported by Nettie Stevens and Edmund Wilson's discovery that sex was associated with a specific chromosome in insects and by Calvin Bridges's demonstration that nondisjunction of X chromosomes was related to the inheritance of eye color in *Drosophila* (see Chapter 4). Further evidence for the chromosome theory of heredity came in 1931, when Harriet Creighton and Barbara McClintock (**Figure 7.8**) obtained evidence that intrachromosomal recombination was the result of physical exchange between chromosomes. Creighton and McClintock discovered a strain of corn that had an abnormal chromosome 9, containing a densely staining knob at one end and a small piece of another chromosome attached to the other end. This aberrant chromosome allowed them to visually distinguish the two members of a homologous pair.

Karl Maramorosch/Courtesy of Cold Spring Harbor Laboratory Archives.

7.8 Barbara McClintock (left) and Harriet Creighton (right) provided evidence that genes are located on chromosomes.

Creighton and McClintock focused on the inheritance of two traits in corn determined by genes on chromosome 9. At one locus, a dominant allele (*C*) produced colored kernels, whereas a recessive allele (*c*) produced colorless kernels. At a second, linked locus, a dominant allele (*Wx*) produced starchy kernels, whereas a recessive allele (*wx*) produced waxy kernels. They obtained a plant that was heterozygous at both loci in repulsion, with the alleles for colored and waxy on the aberrant chromosome and the alleles for colorless and starchy on the normal chromosome:



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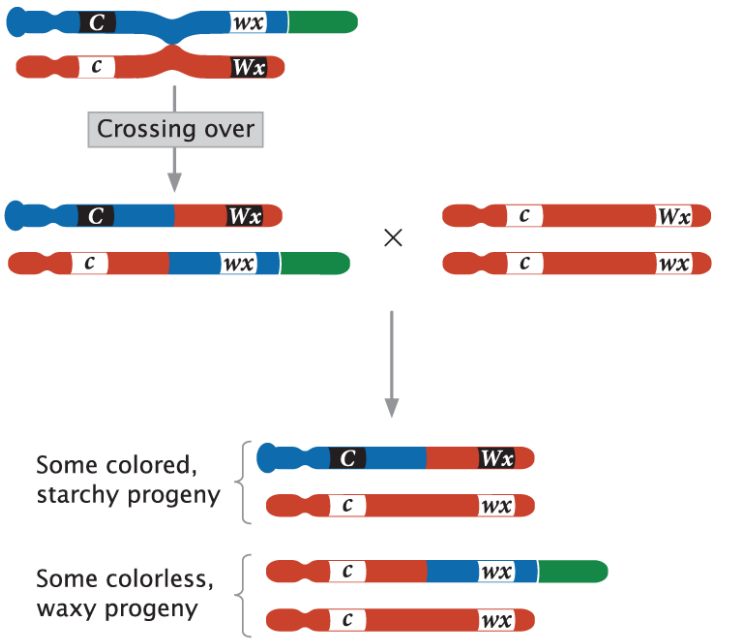
Description
The diagram starts with a drawing of two chromosomes containing the alleles. The top chromosome has a region labeled as knob at the end of the short arm and a region labeled as extra piece (green) at the end of the long arm. The second chromosome does not contain these 2 features. The top chromosome has big C and small W small X alleles on its long arm. The bottom chromosome has small C and big W small X alleles on its long arm.

They then crossed this heterozygous plant with one that was homozygous for colorless and heterozygous for waxy (with both chromosomes normal):

$$\frac{C \quad wx}{c \quad Wx} \times \frac{c \quad Wx}{c \quad wx}$$

This cross produces different combinations of traits in the progeny, but the only way that colorless and waxy progeny can arise is through crossing over in the doubly heterozygous parent:



Note: Not all progeny genotypes are shown.

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Description

A line drawing shows a cross between the two chromosomes. One chromosome has big C and small W small X on top and small C and big W small X on bottom. The chromosome it is crossed with has small C and big W small X on top and small C and small W small X on bottom.

Crossing over occurs between the two chromosomes. The result shows two chromosomes below. The top chromosome has big C with a knob and big W small X and the bottom chromosome has small C and small W small X with the extra piece (green). This is crossed with two normal chromosomes containing alleles, small C and big W small X. The crossover event occurs. The resultant chromosomes are drawn below. One resultant chromosome has the knob on the short arm, big C and big W small X, and normal long arm. The other chromosome has the normal short arm, small C and small W small X. Both these chromosomes are some colored and starchy. The third chromosome has an extra piece (green) on the long arm and alleles small C small W small X and the fourth chromosome has a normal chromosome with alleles, small C and small W small X. Both these chromosomes are labeled colorless, waxy progeny.

A caption below reads, Note: Not all progeny genotypes are shown.

Notice that if crossing over entails physical exchange between the chromosomes, then the colorless, waxy progeny resulting from recombination should have a chromosome with an extra piece, but not a knob. Furthermore, some of the colored, starchy progeny should have a chromosome with a knob, but not the extra piece. This outcome is precisely what Creighton and McClintock observed, confirming the chromosomal theory of inheritance. Curt Stern provided a similar demonstration by using chromosomal markers in *Drosophila* at about the same time. We will examine the molecular basis of recombination in more detail in Chapter 12.

Predicting the Outcomes of Crosses with Linked Genes

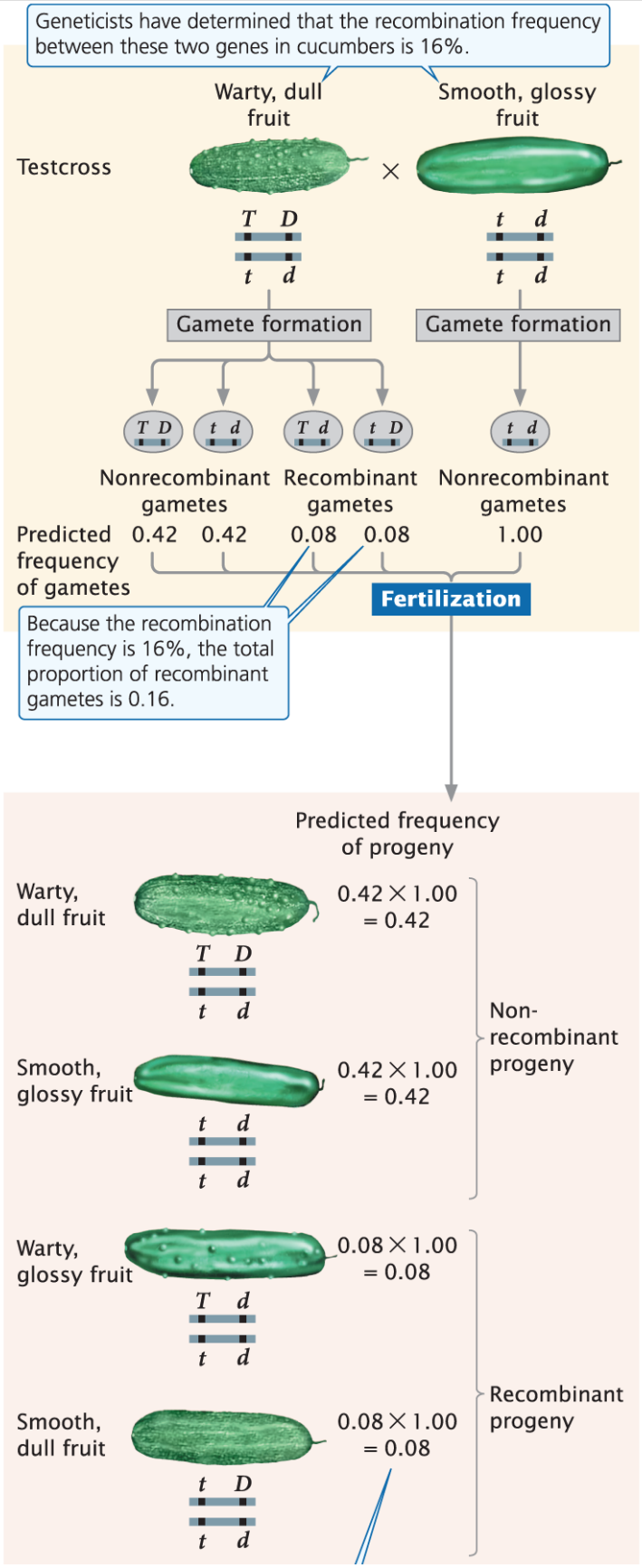
Knowing the arrangement of alleles on a chromosome allows us to predict the types of progeny that will result from a cross entailing linked genes and to determine which of these types will be the most numerous. Determining the *proportions* of the types of offspring requires an additional piece of information: the recombination frequency. The recombination frequency provides us with information about how often the alleles in the gametes appear in new combinations, allowing us to predict the proportions of offspring phenotypes that will result from a specific cross that entails linked genes.

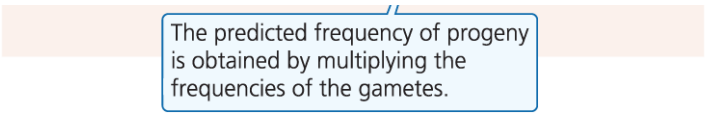
In cucumbers, smooth fruit (*t*) is recessive to warty fruit (*T*), and glossy fruit (*d*) is recessive to dull fruit (*D*). Geneticists have determined that these two genes exhibit a recombination frequency of 16%. Suppose that we cross a plant that is homozygous for warty and dull fruit with a plant that is homozygous for smooth and glossy fruit, and then carry out a testcross using the F_1:

$$\frac{T \quad D}{t \quad d} \times \frac{t \quad d}{t \quad d}$$

What types and proportions of progeny will result from this testcross?

Four types of gametes will be produced by the heterozygous parent, as shown in **Figure 7.9**: two types of nonrecombinant gametes (*T D* and *t d*) and two types of recombinant gametes (*T d* and *t D*). The recombination frequency tells us that 16% of the gametes produced by the heterozygous parent will be recombinants. Because there are two types of recombinant gametes, each should arise with a frequency of $^{16\%}/_{2} = 8\%$. This frequency can also be represented as a probability of 0.08. All the other gametes will be nonrecombinants, so they should arise with a frequency of 100% − 16% = 84%. Because there are two types of nonrecombinant gametes, each should arise with a frequency of $^{84\%}/_{2} = 42\%$ (or 0.42). The other parent in the testcross is homozygous and therefore produces only a single type of gamete (*t d*) with a frequency of 100% (or 1.00).


Geneticists have determined that the recombination frequency between these two genes in cucumbers is 16%.
Warty, dull fruit
Smooth, glossy fruit
Testcross
×
T D
t d
t d
t d
Gamete formation
Gamete formation
T D
t d
T d
t D
t d
Nonrecombinant gametes
Recombinant gametes
Nonrecombinant gametes
Predicted frequency of gametes
0.42 0.42 0.08 0.08 1.00
Fertilization
Because the recombination frequency is 16%, the total proportion of recombinant gametes is 0.16.
Predicted frequency of progeny
Warty, dull fruit
0.42 × 1.00 = 0.42
Smooth, glossy fruit
0.42 × 1.00 = 0.42
Non-recombinant progeny
Warty, glossy fruit
0.08 × 1.00 = 0.08
Smooth, dull fruit
0.08 × 1.00 = 0.08
Recombinant progeny



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7.9 The recombination frequency allows us to predict the proportions of offspring expected for a cross entailing linked genes.

Description

The figure shows a test cross between a warty dull fruit with genotype big T big D small T small D and a smooth glossy fruit with genotype small T small D small T small D. A caption reads, Geneticists have determined that the recombination frequency between 2 genes in cucumbers is 16 percent. All possible gametes are shown, recombinant and nonrecombinant. Below the drawing of the gametes are the predicted frequencies of the gametes.

For warty, dull fruit, gametes formed are as follows:

Nonrecombinant gametes: big T big D and small T small D (predicted frequency 0 point 4 2 and 0 point 4 2 respectively).

Recombinant gametes: big T small D and small T big D (0 point 0 8 and 0 point 0 8 respectively).

A caption reads, Because the recombination frequency is 16 percent, the total proportion of recombinant gametes is 0 point 1 6.

For smooth, glossy fruit:

Nonrecombinant gamete formed: small T small D (1 point 0 0)

Fertilization occurs and F subscript 1 generation is obtainedbelow. The progenies formed are as follows:

Nonrecombinant progeny:

Warty dull fruit, with genotype big T big D small T small D with predicted frequency 0 point 4 2 multiplied by 1 equals 0 point 4 2.

Smooth glossy fruit with genotype small T small D small T small D with predicted frequency 0 point 4 2 multiplied by 1 equals 0 point 4 2.

Recombinant progeny:

Warty, glossy fruit with genotype big T small D small T small D with predicted frequency 0 point 0 8 multiplied by 1 equals 0 point 0 8.

Smooth, dull fruit small T big D small T small D with predicted frequency 0 point 0 8 multiplied by 1 equals 0 point 0 8.

A caption reads, The predicted frequencies of the progeny are obtained by multiplying the frequencies of the gametes.

Four types of progeny result from the testcross (see Figure 7.9). The expected proportion of each progeny type can be determined by using the multiplication rule (see Chapter 3), multiplying together the probability of each gamete. For example, testcross progeny with warty and dull fruit

$$\frac{T \quad D}{t \quad d}$$

appear with a frequency of 0.42 (the probability of inheriting a gamete with chromosome $\underline{T \quad D}$ from the heterozygous parent) × 1.00 (the probability of inheriting a gamete with chromosome $\underline{t \quad d}$ from the recessive parent) = 0.42. The proportions of the other types of F_2 progeny can be calculated in a similar manner (see Figure 7.9). This method can be used for predicting the outcome of any cross with linked genes for which the recombination frequency is known.

THINK-PAIR-SHARE Question 4

Testing for Independent Assortment

In some crosses, it is obvious that the genes are linked because there are clearly more nonrecombinant progeny than recombinant progeny. In other crosses, the difference between independent assortment and linkage isn't as obvious. For example, suppose we did a testcross for two pairs of alleles, such as *Aa Bb* × *aa bb*, and observed the following numbers of progeny: 54 *Aa Bb*, 56 *aa bb*, 42 *Aa bb*, and 48 *aa Bb*. Is this outcome the 1 : 1 : 1 : 1 ratio we would expect if *A* and *B* assorted independently? Not exactly, but it's pretty close. Perhaps these genes assorted independently and chance produced the slight deviations between the observed numbers and the expected 1 : 1 : 1 : 1 ratio. Alternatively, the genes might be linked, but considerable crossing over might be taking place between them, and so the number of nonrecombinants is only slightly greater than the number of recombinants. How do we distinguish between the role of chance and the role of linkage in producing deviations from the results expected with independent assortment?

We encountered a similar problem in crosses in which genes were unlinked: the problem of distinguishing between deviations due to chance and those due to other factors. We addressed this problem (in Chapter 3) with the chi-square goodness-of-fit test, which helps

us evaluate the likelihood that chance alone is responsible for deviations between the numbers of progeny that we observe and the numbers that we expect according to the principles of inheritance. Here, we are interested in a different question: Is the inheritance of alleles at one locus independent of the inheritance of alleles at a second locus? If the answer to this question is yes, then the genes are assorting independently; if the answer is no, then the genes are probably linked.

A possible way to test for independent assortment is to calculate the expected probability of each progeny type, assuming independent assortment, and then use the chi-square goodness-of-fit test to evaluate whether the observed numbers deviate significantly from the expected numbers. With independent assortment, we expect $1/4$ of each phenotype: $1/4$ *Aa Bb*, $1/4$ *aa bb*, $1/4$ *Aa bb*, and $1/4$ *aa Bb*. This expected probability of each genotype is based on the multiplication rule (see Chapter 3). For example, if the probability of *Aa* is $1/2$ and the probability of *Bb* is $1/2$, then the probability of *Aa Bb* is $1/2 \times 1/2 = 1/4$. In this calculation, we are making two assumptions: (1) that the probability of each single-locus genotype is $1/2$, and (2) that genotypes at the two loci are inherited independently $(1/2 \times 1/2 = 1/4)$.

One problem with this approach is that a significant chi-square value can result from a violation of either assumption. If the genes are linked, then the inheritance of genotypes at the two loci is not independent (assumption 2), and we will get a significant deviation

between observed and expected numbers. But we can also get a significant deviation if the probability of each single-locus genotype is not $^1/_2$ (assumption 1), even when the genotypes are assorting independently. We may obtain a significant deviation, for example, if individuals with one genotype have a lower probability of surviving or if the penetrance of a genotype is not 100%. We could test both assumptions by conducting a series of chi-square tests, first testing the inheritance of genotypes at each locus separately (assumption 1) and then testing for independent assortment (assumption 2). However, a faster method is to test for independence in genotypes with a *chi-square test of independence*.

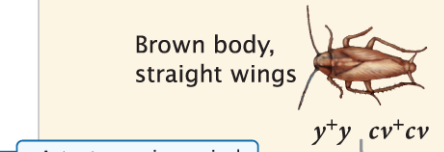
THE CHI-SQUARE TEST OF INDEPENDENCE

The chi-square test of independence allows us to evaluate whether the segregation of alleles at one locus is independent of the segregation of alleles at another locus without making any assumption about the probability of single-locus genotypes. In the chi-square goodness-of-fit test, the expected value is based on a theoretical relation: for example, the expected ratio in a genetic cross. For the chi-square test of independence, the expected value is based strictly on the observed values, along with the assumption that they are independent.

To illustrate this analysis, let's examine the results of a cross between German cockroaches, in which yellow body (*y*) is recessive

to brown body (y^+), and curved wings (cv) are recessive to straight wings (cv^+). A testcross ($y^+y\ cv^+cv \times yy\ cvcv$) produces the progeny shown in **Figure 7.10a**. If the segregation of alleles at each locus is independent, then the proportions of progeny with y^+y and yy genotypes should be the same for cockroaches with genotype cv^+cv and for cockroaches with genotype $cvcv$. The converse is also true: the proportions of progeny with cv^+cv and $cvcv$ genotypes should be the same for cockroaches with genotype y^+y and for cockroaches with genotype yy.

(a)



Brown body, straight wings × Yellow body, curved wings

y^+y cv^+cv × yy $cvcv$

1 A testcross is carried out between cockroaches differing in two characteristics.

Cross

63	y^+y cv^+cv	brown body, straight wings
28	y^+y $cvcv$	brown body, curved wings
33	yy cv^+cv	yellow body, straight wings
77	yy $cvcv$	yellow body, curved wings

(b) Contingency table

2 To test for independent assortment of alleles encoding the two traits, a table is constructed...

Segregation of y^+ and y 3 ...with genotypes for one locus along the top...

Segregation of cv^+ and cv 4 ...and genotypes for the other locus along the left side.

	y^+y	yy	Row totals
cv^+cv	63	33	96
cv cv	28	77	105
Column totals	91	110	201 Grand total

5 Numbers of each genotype are placed in the table cells, and the row totals, column totals, and grand total are computed.

(c)

Genotype	Number observed	Number expected $\left(\frac{\text{row total} \times \text{column total}}{\text{grand total}}\right)$
y^+y cv^+cv	63	$\frac{96 \times 91}{201} = 43.46$
y^+y $cvcv$	28	$\frac{105 \times 91}{201} = 47.54$
yy cv^+cv	33	$\frac{96 \times 110}{201} = 52.54$
yy $cvcv$	77	$\frac{105 \times 110}{201} = 57.46$

6 The expected numbers of progeny, assuming independent assortment, are calculated.

(d)

7 A chi-square value is calculated.

$$\chi^2 = \Sigma \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

$$= \frac{(63 - 43.46)^2}{43.46} + \frac{(28 - 47.54)^2}{47.54} + \frac{(33 - 52.54)^2}{52.54} + \frac{(77 - 57.46)^2}{57.46}$$

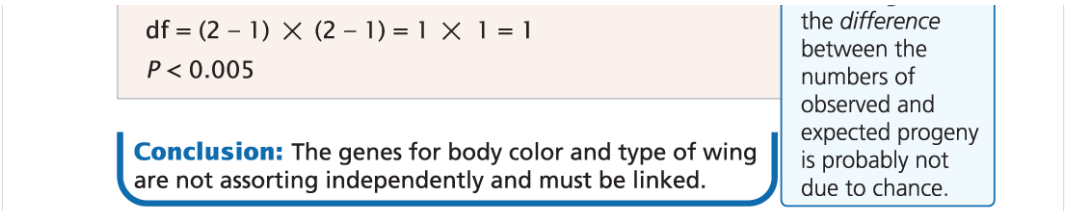
$$= 8.79 + 8.03 + 7.27 + 6.64$$

$$= 30.73$$

(e)

$\text{df} = (\text{number of rows} - 1) \times (\text{number of columns} - 1)$

8 The probability is less than 0.005, indicating that



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7.10 A chi-square test of independence can be used to determine if genes at two loci are assorting independently.

Description

Part a at the top shows a cockroach with brown body, straight wings with genotype small Y superscript positive small Y small C small V superscript positive small C small V crossed with a cockroach with yellow body, curved wings with genotype small Y small Y small C small V small C small V. Cross is conducted and a caption reads, A testcross is carried out between cockroaches differing in two characteristics. In the F subscript 1 generation, there are four types of progeny obtained. 63 have genotype small Y superscript positive small Y small C small V superscript positive small C small V and are brown cockroaches with straight wings. 28 have the genotype small Y small Y superscript positive small C small V small C small V and are brown, curved-wing cockroaches. 33 have genotype small Y small Y small C small V superscript positive small C small V and are yellow, straight-winged cockroaches. 77 have the genotype small Y small Y small C small V small C small V and are yellow curved-winged cockroaches.

Part b below shows a contingency table with 4 columns and 3 rows. A caption reads, To test for independent assortment of alleles encoding the two traits, a table is constructed with genotypes for one locus along the top and genotypes for the other locus along the left side. The headers of the columns represents segregation of small Y superscript positive and small Y and are labeled with each of the genotypes small Y superscript positive small Y and small Y small Y, with the last column header labeled row totals. The row entries are as follows:

Row 1: small C small V superscript positive small C small V; 63; 33; 96.

Row 2: small C small V small C small V; 28; 77; 105.

Row 3: column totals; 91; 110; 201 (grand total).

Another caption reads, Numbers of each genotype are placed in the table cells, and the row totals, column totals, and grand total are computed.

Part c below shows a table with 4 rows and 3 columns. The column headers are as follows: Genotype, number observed, and a formula for number expected as follows: left parenthesis row total multiplied by column total over grand total right parenthesis. The row entries are as follows:

Row 1: small Y superscript positive small Y small C small V superscript positive small C small V; 63; 96 multiplied by 91 over 201 equals 43 point 4 6

Row 2: small Y superscript positive small Y small C small V small C small V; 28; 105 multiplied by 91 over 201 equals 47 point 5 4

Row 3: small Y small Y small C small V superscript positive small C small V; 33; 96 multiplied by 110 over 201 equals 52 point 4 6

Row 4: small Y small Y small C small V small C small V; 77; 105 multiplied by 110 over 201 equals 57 point 4 6

A caption reads, The expected numbers of progeny, assuming independent assortment, are calculated.

Part d below shows calculated chi-square value. Chi square equals sigma start fraction left parenthesis observed minus expected right parenthesis squared over expected end fraction equals start fraction left parenthesis 63 minus 43 point 4 6 right parenthesis squared over 43 point 4 6 end fraction plus start fraction left parenthesis 28 minus 47 point 5 4 right parenthesis squared over 47 point 5 4 end fraction plus left parenthesis 33 minus 52 point 5 4 right parenthesis squared over 52 point 5 4 plus start fraction left parenthesis 77 minus 57 point 4 6 right parenthesis squared over 57 point 4 6 end fraction equals 8 point 7 9 plus 8 point 0 3 plus 7 point 2 7 plus 6 point 6 4 equals 30 point 7 3. A caption reads, A chi-square value is calculated.

Part e below shows calculation for probability. small D small F equals left parenthesis number of rows minus 1 right parenthesis multiplied by left parenthesis number of columns minus 1 right parenthesis. small D small F equals left parenthesis 2 minus 1 right parenthesis multiplied by left parenthesis 2 minus 1 right parenthesis equals 1 multiplied by 1 equals 1. big P is less than 0 point 0 0 5. A caption reads, The probability is less than 0

point 0 0 5, indicating that the difference between numbers of observed and expected progeny is probably not due to chance.

A caption below reads, Conclusion: The genes for body color and type of wing are not assorting independently and must be linked.

To determine whether the proportions of progeny with genotypes at the two loci are independent, we first construct a table of the observed numbers of progeny, somewhat like a Punnett square, except that we put the genotypes that result from the segregation of alleles at one locus along the top and the genotypes that result from the segregation of alleles at the other locus along the side (**Figure 7.10b**). Next, we compute the total for each row, the total for each column, and the grand total (the sum of all row totals or the sum of all column totals, which should be the same). These totals will be used to compute the expected values for the chi-square test of independence.

Our next step is to compute the expected numbers for each combination of genotypes (each cell in the table) under the assumption that the segregation of alleles at the *y* locus is independent of the segregation of alleles at the *cv* locus. If the segregation of alleles at each locus is independent, the expected number for each cell can be computed with the following formula:

$$\text{expected number} = \frac{\text{row total} \times \text{column total}}{\text{grand total}}$$

For the cell of the table corresponding to genotype y^+y cv^+cv (the upper-left cell of the table in Figure 7.10b), the expected number is

$$\frac{96 \text{ (row toal)} \times 91 \text{ (column total)}}{201 \text{ (grand total)}} = \frac{8736}{201} = 43.46$$

The expected numbers for each cell calculated by this method are given in **Figure 7.10c**.

We now calculate a chi-square value by using the same formula that we used for the chi-square goodness-of-fit test in Chapter 3:

$$x^2 = \sum \frac{(\text{observed} - \text{expected})^2}{(\text{expected})}$$

Recall that Σ means "sum" and that we are adding together the (observed−expected)^2/expected values for the four types of progeny. The observed and expected numbers of cockroaches from the testcross give us a calculated chi-square value of 30.73 (**Figure 7.10d**).

To determine the probability associated with this chi-square value, we need the degrees of freedom. Recall from Chapter 3 that the degrees of freedom are the number of ways in which the observed classes are free to vary from the expected values. In general, for the chi-square test of independence, the degrees of freedom equal the number of rows in the table minus 1, multiplied by the number of columns in the table minus 1 (**Figure 7.10e**), or

$$\text{df} = (\text{number of rows} - 1) \times (\text{number of columns} - 1)$$

In our example, there are two rows and two columns, and so the degrees of freedom are

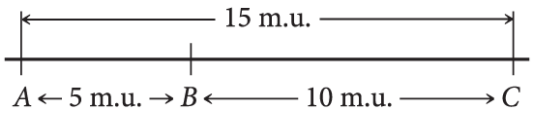
$$\text{df} = (2 - 1) \times (2 - 1) = 1 \times 1 = 1$$

Therefore, our calculated chi-square value is 30.73, with 1 degree of freedom. We can use Table 3.7 to find the associated probability. Looking at Table 3.7, we find that our calculated chi-square value is larger than the largest chi-square value given for 1 degree of freedom, which has a probability of 0.005. Thus, our calculated chi-square value has a probability of less than 0.005. This very small probability indicates that the genotypes are not in the proportions that we would expect if independent assortment were taking place. Our conclusion, then, is that these genes are not assorting independently and must be linked. As in the case of the chi-square goodness-of-fit test, geneticists generally consider that any chi-square value for the test of independence with a probability of less than 0.05 means that the observed values are significantly different from the expected values and is therefore evidence that the genes are not assorting independently. **› TRY PROBLEM 17**

Gene Mapping with Recombination Frequencies

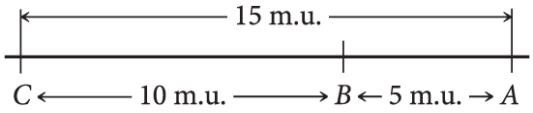
Thomas Hunt Morgan and his students developed the idea that physical distances between genes on a chromosome are related to their rates of recombination. They hypothesized that crossover events take place more or less at random up and down the chromosome and that two genes that lie far apart are more likely to undergo a crossover than are two genes that lie close together. They proposed that recombination frequencies could provide a convenient way to determine the order of genes along a chromosome and would give estimates of the relative distances between the genes. Chromosome maps calculated by using the genetic phenomenon of recombination are called **genetic maps**. In contrast, chromosome maps calculated by using physical distances along the chromosome (often expressed as numbers of base pairs) are called **physical maps**.

Distances on genetic maps are measured in **map units** (abbreviated m.u.); one map unit equals a 1% recombination rate. Map units are also called **centiMorgans (cM)**, in honor of Thomas Hunt Morgan. Genetic distances measured with recombination rates are approximately additive: if the distance from gene *A* to gene *B* is 5 m.u., the distance from gene *B* to gene *C* is 10 m.u., and the distance from gene *A* to gene *C* is 15 m.u., then gene *B* must be located between genes *A* and *C*. On the basis of the map distances just given, we can draw a simple genetic map for genes *A*, *B*, and *C*, as shown here:



Description
A line is drawn and the gene locations are indicated with notches in the line. On the left is big A and in the middle is big B. The distance 5 m u and arrows projecting from right and left is written between big A and big B. Between big B and big C is the distance 10 m u written with arrows projecting right and left. Above the line is written the total distance, 15 m u with arrows extending all the way to big A and big C.

We could just as plausibly draw this map with *C* on the left and *A* on the right:

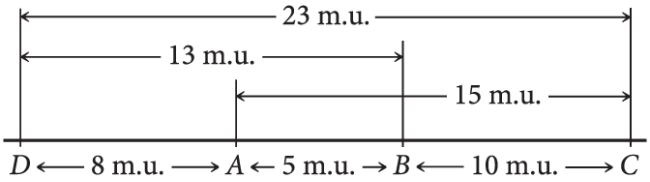


Description
From the left, their sequence is big A, big B, and big C. Distance between big A and big B is 5 m u. Distance between big B and big C is 10 m u. Total distance from big A to big C is 15 m u.

Both maps are correct and equivalent because, with information about the relative positions of only three genes, the most that we can determine is which gene lies in the middle. If we obtained distances to an additional gene, then we could position *A* and *C* relative to that gene. An additional gene *D*, examined through genetic crosses, might yield the following recombination frequencies:

Gene pair	Recombination frequency (%)
A and *D*	8
B and *D*	13
C and *D*	23

Notice that *C* and *D* exhibit the highest rate of recombination; therefore, *C* and *D* must be farthest apart, with genes *A* and *B* between them. Using the recombination frequencies and remembering that 1 m.u. = 1% recombination, we can now add *D* to our map:



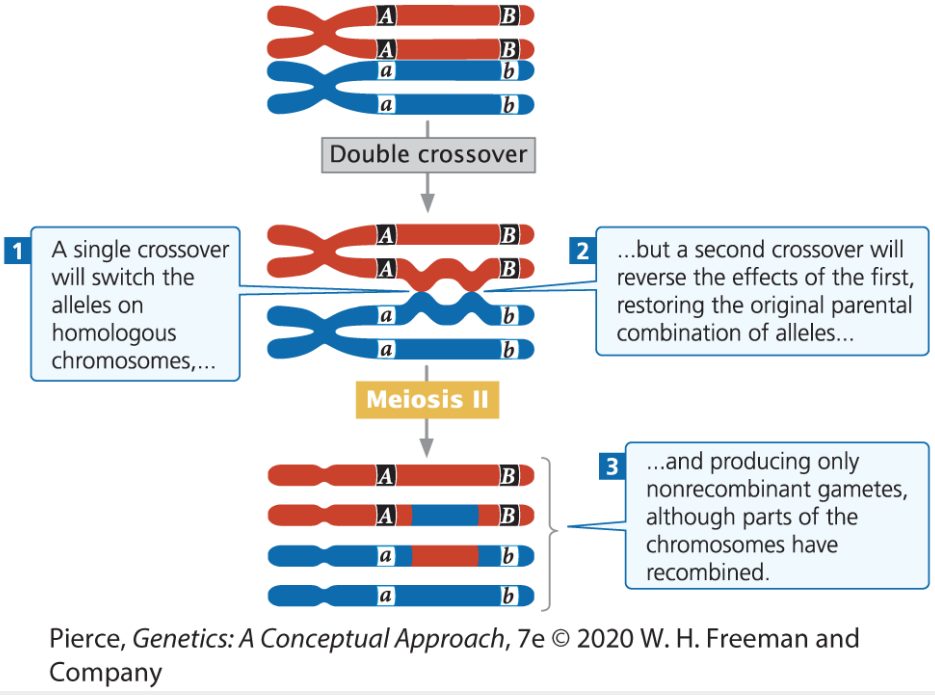
Description
From the left, their sequence is big D, big A, big B, and big C. Distance between big D and big A is 8 m u. Distance between big A and big B is 5 m u. Distance between big B and big C is 10 m u. Distance from big D to big B is 13 m u, from big A to big C is 15 m u, and total distance from big D to big C is 23 m u.

Thus, by doing a series of crosses between pairs of genes, we can construct genetic maps showing the linkage arrangements of a number of genes.

Two points about constructing chromosome maps from recombination frequencies should be emphasized. First, recall that we cannot distinguish between genes on different chromosomes and genes located far apart on the same chromosome. If genes exhibit 50% recombination, the most that can be said about them is that they belong to different linkage groups, either on different chromosomes or far apart on the same chromosome.

The second point is that a testcross for two genes that are far apart on the same chromosome tends to underestimate the true physical distance between them because the cross does not reveal double crossovers that might take place between the two genes (**Figure 7.11**). A double crossover arises when two separate crossover events take place between two loci. (For now, we will consider only double crossovers that take place between two of the four chromatids of a homologous pair—two-strand double crossovers. Double crossovers that take place among three and four chromatids will be considered later, in the section Effects of Multiple Crossovers.) Whereas a single crossover between two genes produces combinations of alleles that were not present on the original parental chromosomes, a second crossover between the same two genes reverses the effects of the first, thus restoring the original parental combination of alleles (see **Figure 7.11**). We therefore cannot distinguish between the progeny produced by two-strand double crossovers and the progeny produced when there is no crossing over at all. As we see in the next section, however, we can detect double crossovers if we examine a third gene that lies between the two crossovers. Because double

crossovers between two genes go undetected, map distances will be underestimated whenever double crossovers take place. Double crossovers are more frequent between genes that are far apart; therefore, genetic maps based on short distances are usually more accurate than those based on longer distances.



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7.11 A two-strand double crossover between two linked genes produces only nonrecombinant gametes.

Description

The diagram shows X-shaped red and blue chromosomes. On the red chromosome, the alleles are big A big B on both the chromatids. On the blue chromosome, the allele are small A small B on both the chromatids. Double crossover occurs and the red and blue chromatids of each chromosome that lie in the middle meet at two points. A caption reads, A single crossover will switch the alleles on homologous chromosomes, but a second crossover will reverse the effects of the first, restoring the original parental combination of allele and producing only nonrecombinant gametes, although parts of the chromosomes have recombined. Meiosis 2 occurs and the resultant chromosomes below are as follows:

Red chromosome with alleles big A and big B.

Red chromosome with alleles big A and big B where a part in between is blue.

Blue chromosome with alleles small A and small B where a part in between is red.

Blue chromosome with alleles small A and small B.

CONCEPTS

Recombination frequencies can be used to determine the order of the genes on a chromosome and calculate the approximate distances from one gene to another. In genetic maps, 1% recombination equals 1 map unit, or 1 centiMorgan. Double crossovers between two genes go undetected, so map distances between distant genes tend to underestimate genetic distances.

✔ CONCEPT CHECK 3

How does a genetic map differ from a physical map?

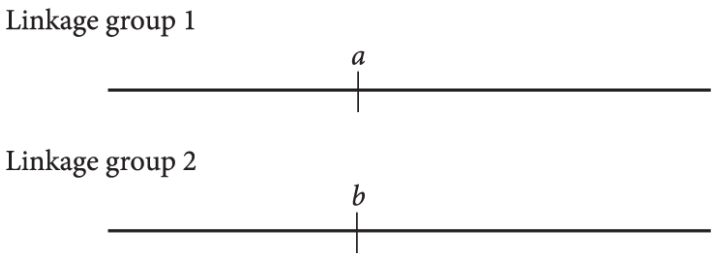
Constructing a Genetic Map with a Two-Point Testcross

Genetic maps can be constructed by conducting a series of testcrosses. In each testcross, one of the parents is heterozygous for a different pair of genes, and recombination frequencies are calculated for each pair of genes. A testcross between two genes is called a **two-point testcross**, or simply a two-point cross. Suppose

that we carried out a series of two-point crosses for four genes, *a*, *b*, *c*, and *d*, and obtained the following recombination frequencies:

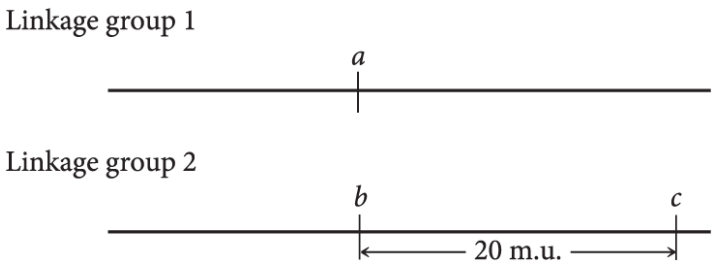
Gene loci in testcross	Recombination frequency (%)
a and *b*	50
a and *c*	50
a and *d*	50
b and *c*	20
b and *d*	10
c and *d*	28

We can begin constructing a genetic map for these genes by considering the recombination frequency for each pair of genes. The recombination frequency between *a* and *b* is 50%, which is the recombination frequency expected with independent assortment. Therefore, genes *a* and *b* may either be on different chromosomes or be very far apart on the same chromosome; we will place them in different linkage groups with the understanding that they may or may not be on the same chromosome:



Description
Linkage group 1 is a line with a notch in the middle labeled gene a. Linkage group 2 is a line with a notch in the middle labeled gene b.

The recombination frequency between *a* and *c* is 50%, indicating that they, too, are in different linkage groups. The recombination frequency between *b* and *c* is 20%, so these genes are linked and separated by 20 map units:

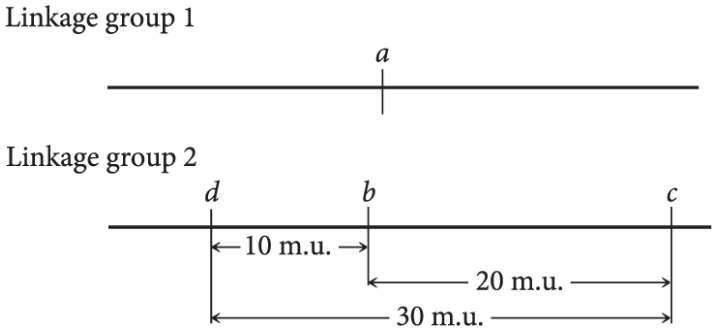


Description
Linkage group 1 is a line with a notch in the middle labeled gene a. Linkage group 2 is a line with a notch in the middle labeled gene b. 20 m u to the right of gene b is a notch labeled c.

The recombination frequency between *a* and *d* is 50%, indicating that these genes belong to different linkage groups, whereas genes *b* and *d* are linked, with a recombination frequency of 10%. To decide whether gene *d* is 10 m.u. to the left or to the right of gene *b*, we

must consult the *c-to-d* distance. If gene *d* is 10 m.u. to the left of gene *b*, then the distance between *d* and *c* should be approximately the sum of the distance between *b* and *c* and between *c* and *d*: 20 m.u.+10 m.u.=30 m.u. If, on the other hand, gene *d* lies to the right of gene *b*, then the distance between gene *d* and gene *c* will be much shorter, approximately 20 m.u.−10 m.u.=10 m.u. Again, the summed distances will be only approximate because any double crossovers between the two genes will be missed and the map distance will be underestimated.

By examining the recombination frequency between *c* and *d*, we can distinguish between these two possibilities. The recombination frequency between *c* and *d* is 28%, so gene *d* must lie to the left of gene *b*. Notice that the sum of the recombination frequency between *d* and *b* (10%) and between *b* and *c* (20%) is greater than the recombination frequency between *d* and *c* (28%). As already discussed, this discrepancy arises because double crossovers between the two outer genes go undetected, causing an underestimation of the true map distance. The genetic map of these genes is now complete:



Description

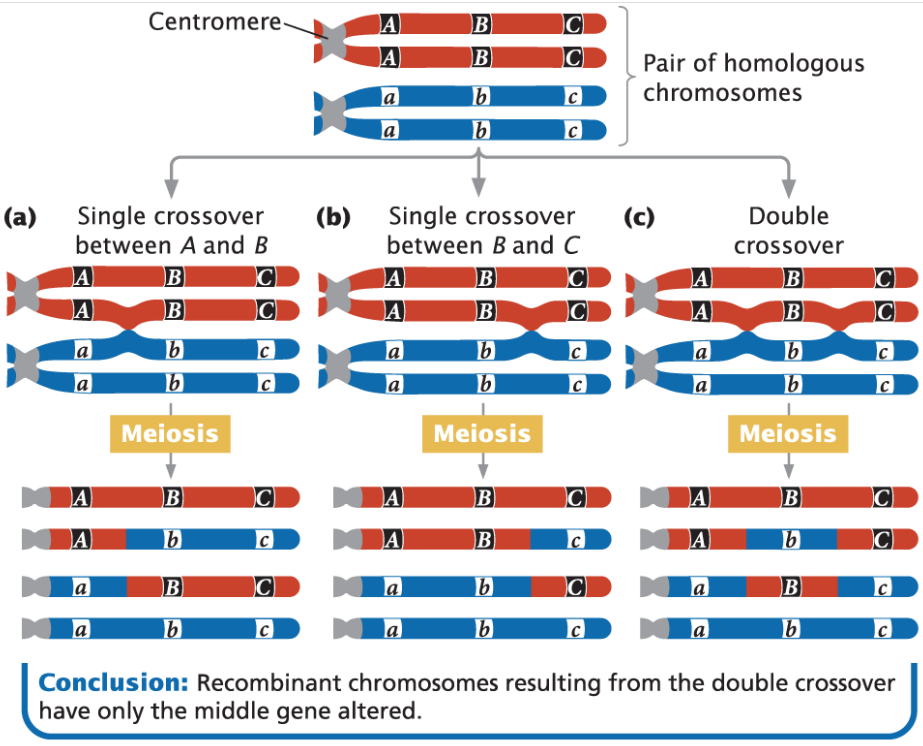
Linkage group 1 is a line with a notch in the middle labeled gene a. Linkage group 2 is a line with a notch in the middle labeled gene b. 20 m u to the right of gene b is gene c, and 10 m u to the left of gene b is gene d.

› **TRY PROBLEM 28**

7.3 A Three-Point Testcross Can Be Used to Map Three Linked Genes

While genetic maps can be constructed from a series of testcrosses for pairs of genes, this approach is not particularly efficient, because numerous two-point crosses must be carried out to establish the order of the genes and because double crossovers will be missed. A more efficient mapping technique is a testcross for three genes—a **three-point testcross**, or three-point cross. With a three-point cross, the order of the three genes can be established in a single set of progeny, and some double crossovers can usually be detected, providing more accurate map distances.

Consider what happens when crossing over takes place among three hypothetical linked genes. **Figure 7.12** illustrates a pair of homologous chromosomes of an individual that is heterozygous at three loci (*Aa Bb Cc*). Notice that the genes are in the coupling configuration: all the dominant alleles are on one chromosome ($\underline{A\ \ B\ \ C}$) and all the recessive alleles are on the other chromosome ($\underline{a\ \ b\ \ c}$). Three types of crossover events can take place between these three genes: two types of single crossovers (see Figure 7.12a and b) and a double crossover (see Figure 7.12c). In each type of crossover, two of the resulting chromosomes are recombinants and two are nonrecombinants.



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7.12 Three types of crossovers can take place among three linked loci.

Description

A pair of homologous chromosomes is at the top. The red chromosome has big A big B big C alleles on both the chromatids. The blue chromosome has small A small B small C alleles on both the chromatids. There are 3 arrows pointing downward showing three different types of crossovers.

Part a on the left shows single crossover between big A and big B, part b shows single crossover between big B and big C, and part c shows double crossover.

Part a shows the chromosomes crossing over at 1 point between big A and big B. Meiosis occurs and 4 resultant chromosomes are formed below as follows: red chromosome with big A big B big C; red chromosome with big A and blue part that has small B small C; blue chromosome with small A and red part that has big B big C; and a blue chromosome with small A small B small C.

Part b in the middle shows red and blue chromosomes crossing over at 1 point between big B and big C. Meiosis occurs and 4 resultant chromosomes are formed below as follows: red

chromosome with big A big B big C; red chromosome with big A big B and blue part that has small C; blue chromosome with small A small B and red part that has big C; and a blue chromosome with small A small B small C.

Part c on the right shows double crossover between red and blue chromosomes such that they are touching at 2 points between the three genes. Meiosis occurs and 4 resultant chromosomes are formed below as follows: red chromosome with big A big B big C; red chromosome with big A, blue part with small B, red part with big C; blue chromosome with small A, red part with big B, blue part with small C; and a blue chromosome with small A small B small C.

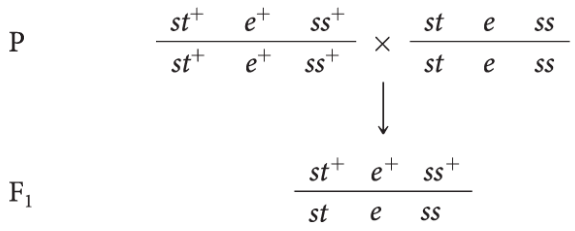
Notice that in the recombinant chromosomes resulting from the double crossover, the outer two alleles are the same as in the nonrecombinants, but the middle allele is different. This result provides us with an important clue about the order of the genes. In progeny that result from a double crossover, only the middle allele should differ from the alleles present in the nonrecombinant progeny.

Constructing a Genetic Map with a Three-Point Testcross

To examine gene mapping with a three-point testcross, let's consider three recessive mutations in the fruit fly *Drosophila melanogaster*. In this species, scarlet eyes (*st*) are recessive to wild-type red eyes (st^+), ebony body color (*e*) is recessive to wild-type gray body color (e^+), and spineless (*ss*)—that is, the presence of small bristles—is recessive to wild-type normal bristles (ss^+). The loci encoding these three characteristics are linked and located on chromosome 3.

We will refer to these three loci as *st*, *e*, and *ss*, but keep in mind that either the recessive alleles (*st*, *e*, and *ss*) or the dominant alleles (st^+, e^+, and ss^+) may be present at each locus. So when we say that there are 10 m.u. between *st* and *ss*, we mean that there are 10 m.u. between the loci at which mutations *st* and *ss* occur; we could just as easily say that there are 10 m.u. between st^+ and ss^+.

To map these genes, we need to determine their order on the chromosome and the genetic distances between them. First, we must set up a three-point testcross: a cross between a fly heterozygous at all three loci and a fly homozygous for recessive alleles at all three loci. To produce flies heterozygous for all three loci, we might cross a stock of flies that are homozygous for wild-type alleles at all three loci with flies that are homozygous for recessive alleles at all three loci:



Description
The P generation above shows one parent with alleles small S small T superscript positive, small E superscript positive, and small S small S superscript positive on the top and bottom of the line. The other parent has a small S small T, small E, and small S small S on the top and bottom of the line. The F subscript 1 progeny below has the following orientation: small S small T superscript positive, small E superscript positive, and small S small S superscript positive written on the top and a small S small T allele, small E allele, and a small S small S allele on the bottom.

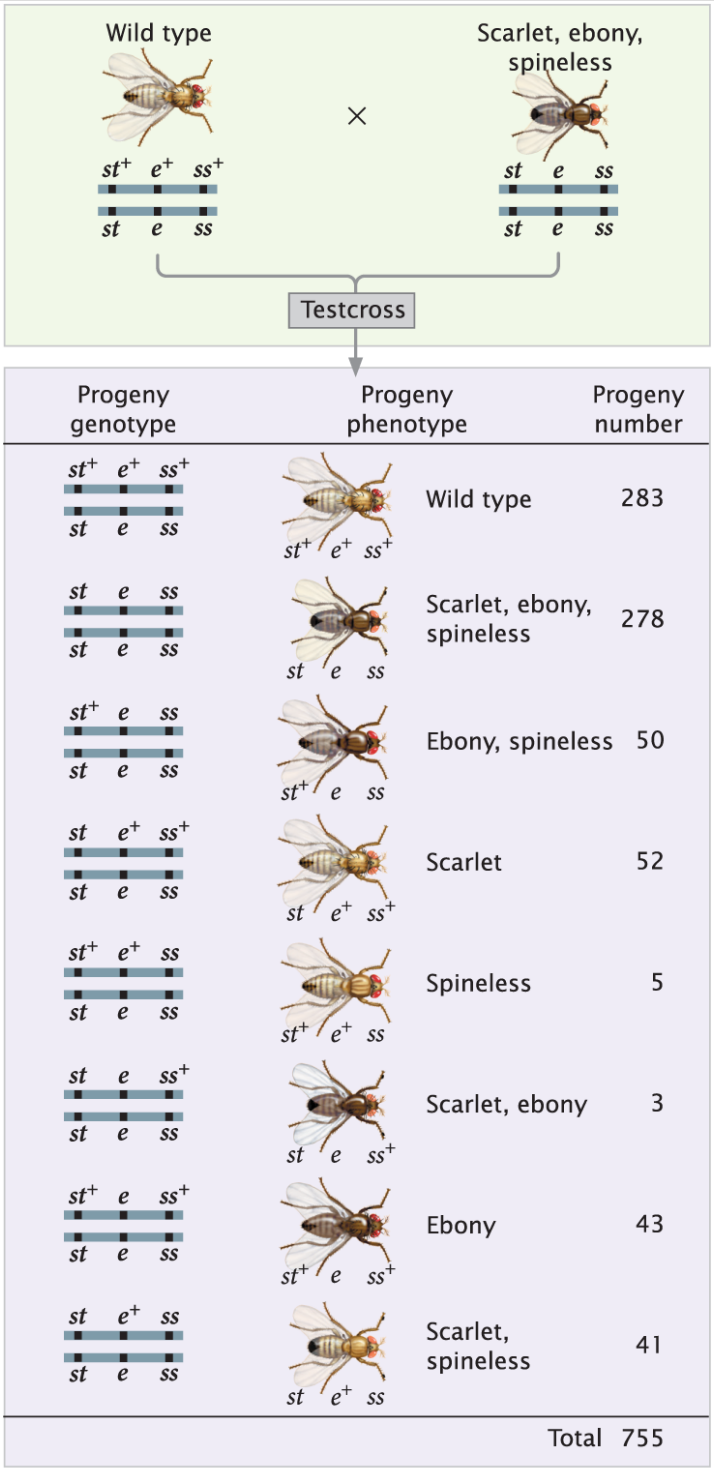
The order of the genes has been arbitrarily assigned because at this point, we do not know which one is the middle gene. Additionally, the alleles in the F_1 heterozygotes are in coupling configuration (because all the wild-type dominant alleles were inherited from one parent and all the recessive mutations from the other parent), although the testcross can also be done with alleles in repulsion.

In the three-point testcross, we cross the F_1 heterozygotes with flies that are homozygous for all three recessive mutations. In many organisms, it makes no difference whether the heterozygous parent in the testcross is male or female (provided that the genes are autosomal), but in *Drosophila*, no crossing over takes place in males. Because crossing over in the heterozygous parent is essential for determining recombination frequencies, the heterozygous flies in our testcross must be female. So we mate female F_1 flies that are heterozygous for all three traits with male flies that are homozygous for all the recessive traits:

$$\frac{st^+ \quad e^+ \quad ss^+}{st \quad e \quad ss}\ \text{Female} \times \frac{st \quad e \quad ss}{st \quad e \quad ss}\ \text{Male}$$

The progeny of this cross are listed in **Figure 7.13**. For each locus, two classes of progeny are produced: progeny that are heterozygous, displaying the dominant trait, and progeny that are homozygous, displaying the recessive trait. With two classes of progeny possible for each of the three loci, there will be $2^3 = 8$ classes of phenotypes possible in the progeny. In this example, all eight phenotypic classes are present, but in some three-point crosses, one or more of the

phenotypes may be missing if the number of progeny is limited. Nevertheless, the absence of a particular class can provide important information about which combination of traits is least frequent and, ultimately, about the order of the genes, as we will see.



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7.13 The results of a three-point testcross can be used to map linked genes. In this three-point testcross of *Drosophila melanogaster*, the recessive mutations leading to scarlet eyes (*st*), ebony body color (*e*), and spineless bristles (*ss*) are at three

linked loci. The order of the loci has been assumed arbitrarily. Each phenotypic class includes both male and female flies; the sex of the pictured flies is random.

Description

The figure shows P generation at the top. A wild type fly has genes small S small T superscript positive, small E superscript positive, and small S small S superscript positive on one top and small S small T, small E, and small S small S on the other chromosome. This fly is crossed with a scarlet, ebony, spineless fly, which has small S small T, small E, and small S small S on both the chromosomes.

Testcross occurs and F subscript 1 generation is obtained below. A table shows the results and has 8 rows and 3 columns. The column headers are as follows: progeny genotype, progeny phenotype, and progeny numbers. The row entries are as follows:

Row 1: small S small T superscript positive, small E superscript positive, and small S small S superscript positive on one chromosome and small S small T small E small S small S on other; wild type; 283.

Row 2: small S small T small E small S small S on both the chromosomes; All mutant; 278.

Row 3: small S small T superscript positive small E small S small S on one chromosome and small S small T small E small S small S on other; ebony, spineless; 50.

Row 4: small S small T small E superscript positive small S small S superscript positive on one chromosome and small S small T small E small S small S on other ; scarlet; 52.

Row 5: small S small T superscript positive small E superscript positive small S small S on one chromosome and small S small T small E small S small S on other; spineless; 5.

Row 6: small S small T small E small S small S superscript positive on one chromosome and small S small T small E small S small S on other; scarlet, ebony; 3.

Row 7: small S small T superscript positive small E small S small S superscript positive on one chromosome and small S small T small E small S small S on other; ebony; 43.

Row 8: small S small T small E superscript positive small S small S on one chromosome and small S small T small E small S small S on other chromosome; scarlet, spineless; 41.

A text below reads, total equals 755.

To map the genes, we need information about where and how often crossing over has taken place. In the homozygous recessive parent, the two alleles at each locus are the same, and so crossing over will have no effect on the types of gametes produced: with or without crossing over, all gametes from this parent have a chromosome with three recessive alleles ($\underline{st\quad e\quad ss}$). In contrast, the heterozygous parent has different alleles on its two chromosomes, and so crossing over can be detected. The information that we need for mapping, therefore, comes entirely from the gametes produced by the heterozygous parent. Because chromosomes contributed by the homozygous parent carry only recessive alleles, whatever alleles are present on the chromosome contributed by the heterozygous parent will be expressed in the progeny.

As a shortcut, we often do not write out the complete genotypes of the testcross progeny, listing instead only the alleles expressed in the phenotype, which are the alleles inherited from the heterozygous parent. This convention is used in the discussion that follows.

CONCEPTS

To map genes, information about the location and number of crossovers in the gametes that produced the progeny of a cross is needed. An efficient way to obtain this information is to use a three-point testcross, in which an individual that is heterozygous at three linked loci is crossed with an individual that is homozygous recessive at the same three loci.

✔ CONCEPT CHECK 4

Write the genotypes of all recombinant and nonrecombinant progeny expected from the following three-point cross:

$$\frac{m^+ \quad p^+ \quad s^+}{m \quad p \quad s} \times \frac{m \quad p \quad s}{m \quad p \quad s}$$

Description
Each chromosome is represented by a straight, horizontal line. For first chromosome: the alleles above the line are small M superscript positive small P superscript positive small S superscript positive and alleles below the line are small M small P small S.

For second chromosome: the alleles above and below the line are small M small P small S. A cross sign is present between the chromosomes.

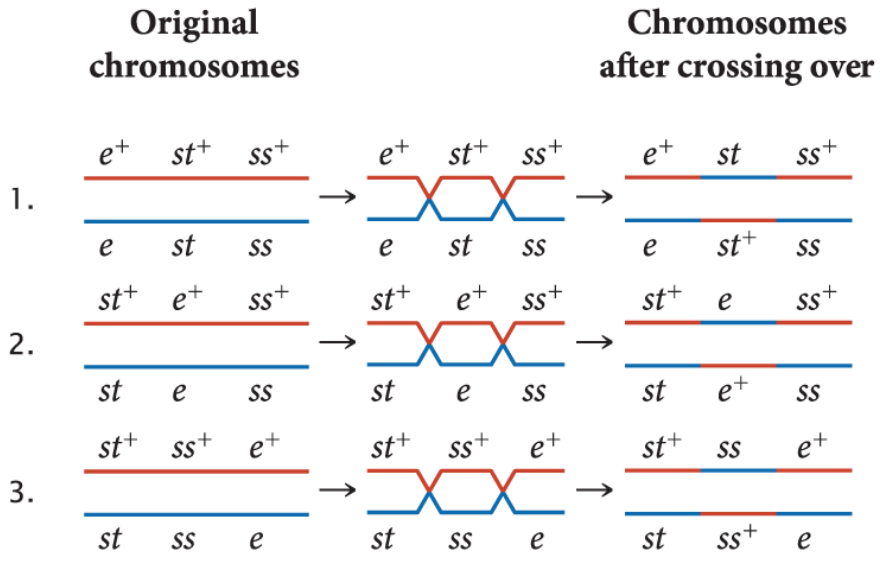
DETERMINING THE GENE ORDER

The first task in mapping the genes is to determine their order on the chromosome. In Figure 7.13, we arbitrarily listed the loci in the order *st*, *e*, *ss*, but we had no way of knowing which of the three loci was between the other two. We can now identify the middle locus by examining the double-crossover progeny.

First, determine which progeny are the nonrecombinants: they will be the two most numerous classes of progeny (even if crossing over takes place in every meiosis, the nonrecombinants will constitute at least 50% of the progeny). Among the progeny of the testcross in Figure 7.13, the most numerous are those with all three dominant traits ($\frac{st^+ \quad e^+ \quad ss^+}{}$) and those with all three recessive traits ($\frac{st \quad e \quad ss}{}$).

Next, identify the double-crossover progeny. These progeny should always have the two least numerous phenotypes because the probability of a double crossover is always less than the probability of a single crossover. The least common progeny among those listed in **Figure 7.13** are progeny with spineless bristles ($\underline{st^+ \quad e^+ \quad ss}$) and progeny with scarlet eyes and ebony body ($\underline{st \quad e \quad ss^+}$), so they are the double-crossover progeny.

Three orders of genes on the chromosome are possible: the eye-color locus could be in the middle ($\underline{e \quad st \quad ss}$), the body-color locus could be in the middle ($\underline{st \quad e \quad ss}$), or the bristle locus could be in the middle ($\underline{st \quad ss \quad e}$). To determine which gene is in the middle, we can draw the chromosomes of the heterozygous parent with all three possible gene orders and then see if a double crossover produces the combination of genes observed in the double-crossover progeny. The three possible gene orders and the types of progeny produced by their double crossovers are



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Description

The diagram shows 3 pairs of original chromosomes, crossing over events, and chromosomes

after crossing over.

First pair has small E superscript positive, small S small T superscript positive, and small S small S superscript positive on the red chromosome and small E, small S small T, and small S small S on the blue chromosome. Crossing over takes place at 2 points. The resultant chromosome has red chromosome with small E superscript positive, blue part with small S small T, and red part small S small S superscript positive. Blue chromosome has small E, red part with small S small T superscript positive, and blue part with small S small S.

Second pair has small S small T superscript positive, small E superscript positive, and small S small S superscript positive on the red chromosome and small S small T, small E, and small S small S on the blue chromosome. Crossing over takes place at 2 points. The resultant chromosome has red chromosome with small S small T superscript positive, blue part with small E, and red part with small S small S superscript positive. Blue chromosome has small S small T, red part with small E superscript positive, and blue part with small S small S.

Third part has small S small T superscript positive, small S small S superscript positive, and small E superscript positive on the red chromosome and small S small T, small S small S,

and small E on the blue chromosome. Crossing over takes place at 2 points. The resultant chromosome has red chromosome with small S small T superscript positive, blue part with small S small S, and red part with small E superscript positive. Blue chromosome has small S small T, red part with small S small S superscript positive, and blue part with small E.

The only gene order that produces chromosomes with the set of alleles observed in the least numerous progeny or double crossovers ($\underline{st^+ \quad e^+ \quad ss}$ and $\underline{st \quad e \quad ss^+}$ in Figure 7.13) is the one in which the *ss* locus for bristles lies in the middle (gene order 3). Therefore, this order ($\underline{st \quad ss \quad e}$) must represent the correct sequence of genes on the chromosome.

With a little practice, we can quickly determine which locus is in the middle without writing out all the gene orders. The phenotypes of the progeny are expressions of the alleles inherited from the heterozygous parent. Recall that when we looked at the results of double crossovers (see Figure 7.12), only the alleles at the middle locus differed from those in the nonrecombinants. If we compare the nonrecombinant progeny with the double-crossover progeny, they should differ only in alleles at the middle locus (**Table 7.2**).

TABLE 7.2 Steps in determining gene order in a three-point cross

1. Identify the nonrecombinant progeny (two most numerous phenotypes).
2. Identify the double-crossover progeny (two least numerous phenotypes).
3. Compare the phenotypes of double-crossover progeny with the phenotypes of nonrecombinant progeny. They should be alike in two characteristics and differ in one.
4. The characteristic that differs between the double crossover and the nonrecombinant progeny is encoded by the middle gene.

Let's compare the alleles in the double-crossover progeny $\underline{st^+ \quad e^+ \quad ss}$ with those in the nonrecombinant progeny $\underline{st^+ \quad e^+ \quad ss^+}$. We see that both have an allele for red eyes (st^+) and both have an allele for gray body (e^+), but the nonrecombinants have an allele for normal bristles (ss^+), whereas the double crossovers have an allele for spineless bristles (ss). Because the bristle locus is the only one that differs between these two types of progeny, it must lie in the middle. We would obtain the same results if we compared the other class of double-crossover progeny ($\underline{st \quad e \quad ss^+}$) with the other class of nonrecombinant progeny ($\underline{st \quad e \quad ss}$). Again, the only locus that differs is the one for bristles. Don't forget that the nonrecombinants and the double crossovers should differ at only one locus; if they differ at two loci, the wrong classes of progeny are being compared. (A) **Animation 7.1** illustrates how to determine the order of three linked genes.

CONCEPTS

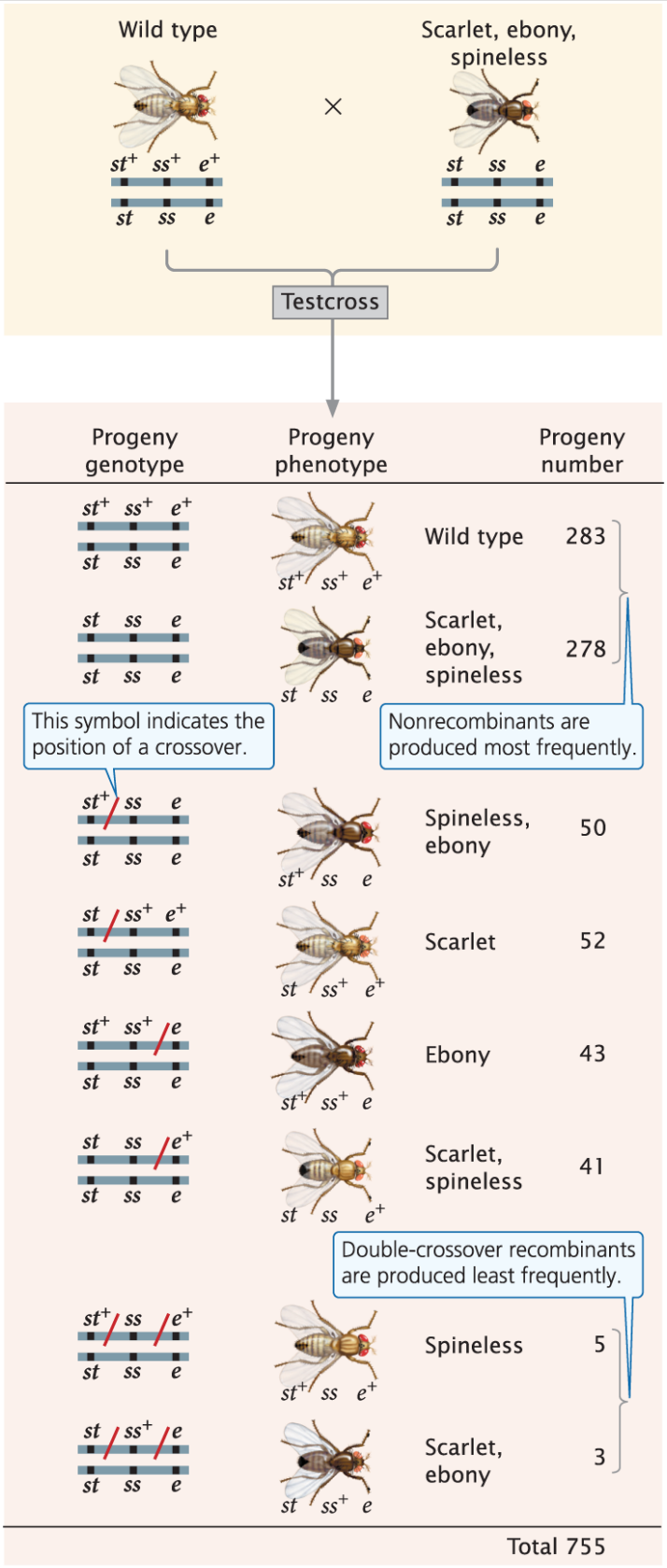
To determine the middle locus in a three-point cross, compare the double-crossover progeny with the nonrecombinant progeny. The double crossovers will be the two least common classes of phenotypes; the nonrecombinants will be the two most common classes of phenotypes. The double-crossover progeny should have the same alleles as the nonrecombinant types at two loci and different alleles at the locus in the middle.

✔ CONCEPT CHECK 5

A three-point testcross is carried out between three linked genes. The resulting nonrecombinant progeny are $s^+ r^+ c^+$ and $s\ r\ c$, and the double-crossover progeny are $s\ r\ c^+$ and $s^+ r^+ c$. Which is the middle locus?

DETERMINING THE LOCATIONS OF CROSSOVERS

When we know the correct order of the loci on the chromosome, we can rewrite the phenotypes of the testcross progeny in Figure 7.13 with the alleles in the correct order so that we can determine where crossovers have taken place (**Figure 7.14**).



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7.14 Writing the results of a three-point testcross with the loci in the correct order allows the locations of crossovers to be determined. These results are from the testcross illustrated in Figure 7.13, with the loci shown in the correct order. The location of a crossover is indicated by a slash (/). Each phenotypic class includes both male and female flies; the sex of the pictured flies is random.

Description

The figure shows P generation. A wild type fly has alleles small S small T superscript positive, small S small S superscript positive, small E superscript positive on one chromosome and small S small T, small S small S, small E on the other chromosome. This fly is crossed with a scarlet, ebony, spineless fly, which has small S small T, small S small S, and small E on both the chromosomes. Testcross occurs and F subscript 1 generation is obtained. A table shows the results and has 8 rows and 3 columns. The column headers are as follows: progeny genotype, progeny phenotype, and progeny numbers. The row entries are as follows:

Row 1: small S small T superscript positive small S small S superscript positive small E superscript positive on one chromosome and small S small T small S small S small E on other; wild type; 283.

Row 2: small S small T small S small S small E on both the chromosomes; Scarlet,

ebony, spineless; 278.

A caption reads, Nonrecombinants are produced most frequently.

Row 3: small S small T superscript positive forward slash small S small S small E on one chromosome and small S small T small S small S small E on other; spineless, ebony; 50.

A caption pointing towards slash reads, This symbol indicates the position of a crossover.

Row 4: small S small T forward slash small S small S superscript positive small E superscript positive on one chromosome and small S small T small S small S small E on other; scarlet; 52.

Row 5: small S small T superscript positive small S small S superscript positive forward slash small E on one chromosome and small S small T small S small S small E on other; ebony; 43.

Row 6: small S small T small S small S forward slash small E superscript positive on one chromosome and small S small T small S small S small E on other; scarlet, spineless; 41.

Row 7: small S small T superscript positive forward slash small S small S forward slash small E superscript positive on one chromosome and small S small T small S small S small E on other; spineless; 5.

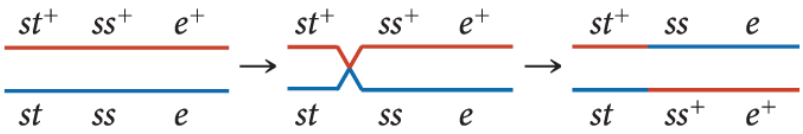
Row 8: small S small T forward slash small S small S superscript positive forward slash small E on one chromosome and small S small T small S small S small E on other chromosome; scarlet, ebony; 3.

A caption reads, Double-crossover recombinants are produced least frequently.

A text below reads, total 755.

Among the eight classes of progeny, we have already identified two classes as nonrecombinants ($\underline{st^+ \quad ss^+ \quad e^+}$ and $\underline{st \quad ss \quad e}$) and two classes as double crossovers ($\underline{st^+ \quad ss \quad e^+}$ and $\underline{st \quad ss^+ \quad e}$). The other four classes contain progeny that resulted from a chromosome that underwent a single crossover: two underwent single crossovers between *st* and *ss*, and two underwent single crossovers between *ss* and *e*.

To determine where the crossovers took place in these single-crossover progeny, we can compare the alleles found in these progeny with those found in the nonrecombinants, just as we did for the double crossovers. For example, consider the progeny with chromosome $\underline{st^+ \quad ss \quad e}$. The first allele ($st^+$) came from the nonrecombinant chromosome $\underline{st^+ \quad ss^+ \quad e^+}$, and the other two alleles (*ss* and *e*) must have come from the other nonrecombinant chromosome $\underline{st \quad ss \quad e}$ through crossing over:



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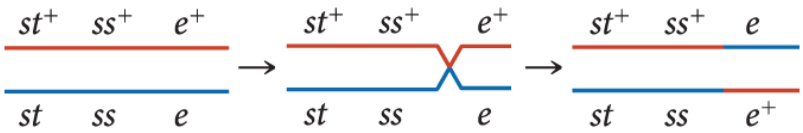
Description
The diagram shows a pair of chromosomes. Red chromosome has small S small T superscript positive, small S small S superscript positive, small E superscript positive. The blue chromosome has small S small T, small S small S, small E. Crossing over occurs at 1 point between the first two genes. The resultant chromosome has red chromosome with small S small T superscript positive, blue part with small S small S, and small E. Blue chromosome has small S small T, red part with small S small S superscript positive, and small E superscript positive.

This same crossover also produces the $st \quad ss^+ \quad e^+$ progeny.

This method can also be used to determine the location of crossing over in the other two types of single-crossover progeny. Crossing over between *ss* and *e* produces ($st^+ \quad ss^+ \quad e$ and $st \quad ss \quad e^+$) chromosomes:



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Description
The diagram shows a pair of chromosome. Red chromosome has small S small T superscript positive, small S small S superscript positive, and small E superscript positive. Blue chromosome has small S small T, small S small S, and small E. Crossing over occurs at 1 point between the last two genes. The resultant chromosome has red chromosome

small S small T superscript positive, small S small S superscript positive, and blue part with small E. The blue chromosome has small S small T, small S small S, and red part with small E superscript positive.

We now know the locations of all the crossovers, which are marked with red slashes in Figure 7.14.

CALCULATING THE RECOMBINATION FREQUENCIES

Next, we can determine the map distances, which are based on the frequencies of recombination. We can calculate recombination frequency by adding up all the recombinant progeny, dividing this number by the total number of progeny from the cross, and multiplying the number obtained by 100%. To determine the map distances accurately, we must include all crossovers (both single and double) that take place between two genes.

Recombinant progeny that possess a chromosome that underwent crossing over between the eye-color locus (*st*) and the bristle locus (*ss*) include the single crossovers ($\underline{st^+ \;/\; ss \;/ e}$ and $\underline{st \;/\; ss^+ \quad e^+}$) and the two double crossovers ($\underline{st^+ \;/\; ss \;/\; e^+}$ and $\underline{st \;/\; ss^+ \;/\; e}$) (see Figure 7.14). There are a total of 755 progeny, so the recombination frequency between *ss* and *st* is:

$$st - ss \text{ recombination frequency} = \frac{50 + 52 + 5 + 3}{755} \times 100\% = 14.6\%$$

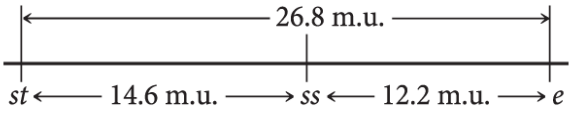
The distance between the *st* and *ss* loci can be expressed as 14.6 m.u.

The map distance between the bristle locus (*ss*) and the body-color locus (*e*) is determined in the same manner. The recombinant progeny that possess a crossover between *ss* and *e* are the single crossovers $\underline{st^+ \ ss^+ \ / \ e}$ and $\underline{st \ ss \ / \ e^+}$ and the double crossovers $\underline{st^+ \ / \ ss \ / \ e^+}$ and $\underline{st \ / \ ss^+ \ / \ e}$. The recombination frequency is

$$ss - e \text{ recombination frequency} = \frac{43 + 41 + 5 + 3}{755} \times 100\% = 12.2\%$$

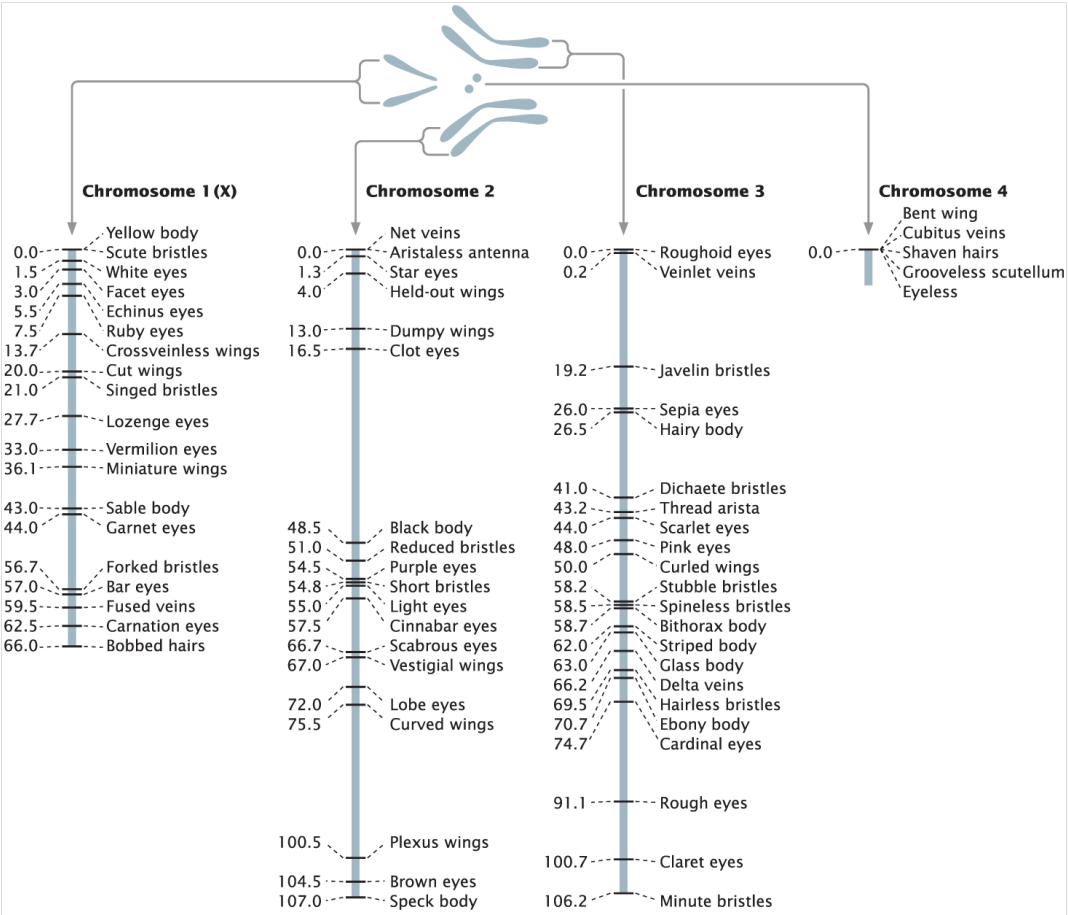
Thus, the map distance between *ss* and *e* is 12.2 m.u.

Finally, calculate the map distance between the outer two loci, *st* and *e*. This map distance can be obtained by summing the map distances between *st* and *ss* and between *ss* and *e* (14.6 m.u. + 12.2 m.u. = 26.8 m.u.). We can now use the map distances to draw a map of the three genes on the chromosome:



Description
Gene small S small T is on the extreme left and 14 point 6 m u to the right is gene small S small S. 12 point 2 m u to the right of gene small S small S is gene small E. The distance between gene small S small T to gene small E is 26 point 8 m u.

A genetic map of *D. melanogaster* is illustrated in Figure 7.15.



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7.15 *Drosophila melanogaster* has four linkage groups corresponding to its four pairs of chromosomes. These genes were mapped using recombination frequencies. Distances between genes within a linkage group are in map units. Note that the small chromosome 4 never undergoes recombination.

Description

The diagram shows the following information:

Chromosome 1 (X): gene at 0 point 0 m u, produces yellow body and scute bristles; 1 point 5, White eyes; 3 point 0, Facet eyes; 5 point 5, Echinus eyes; 7 point 5, Ruby eyes; 13 point 7, Cross vein less wings; 20 point 0, cut wings; 21 point 0, singled bristles; 27 point 7, Lozenge eyes; 33 point 0, Vermilion eyes; 36 point 1, Miniature wings; 43 point 0, Sable body; 44 point 0, Garnet eyes; 56 point 7, Forked bristles; 57 point 0, Bar eyes; 59 point 5, Fused veins; 62 point 5, Carnation eyes; and 66 point 0, Bobbed hairs.

Chromosome 2: 0 point 0, net veins and Aristaless antenna; 1 point 3, Star eyes; 4 point 0, Held-out wings; 13 point 0, Dumpy wings; 16 point 5, Clot eyes; 48 point 5, black body; 51 point 0, Reduced bristles; 54 point 5, Purple eyes; 54 point 8, Short bristles; 55 point 0, Light eyes; 57 point 5, Cinnabar eyes; 66 point 7, Scabrous eyes; 67 point 0, Vestigial wings; 72 point 0, Lobe eyes; 75 point 5, Curved wings; 100 point 5, Plexus wings; 104 point 5, Brown eyes; 107 point 0, Speck body.

Chromosome 3: 0 point 0, Roughoid eyes; 0 point 2, Veinlet veins; 19 point 2, Javelin bristles; 26 point 0, Sepia eyes; 26 point 5, Hairy body; 41 point 0, Dichaete bristles; 43 point 2, Thread arista; 44 point 0, Scarlet eyes; 48 point 0, Pink eyes; 50 point 0, Curled wings; 58 point 2, Stubble bristles; 58 point 5, Spineless bristles; 58 point 7, Bithorax body; 62 point 0, Striped body; 63 point 0, Glass body; 66 point 2, Delta veins; 69 point 5, Hairless bristles; 70 point 7, Ebony body; 74 point 7, Cardinal eyes; 91 point 1, Rough eyes; 100 point 7, Claret eyes; and 106 point 2, Minute bristles.

Chromosome 4: 0 point 0, Bent wing, Cubitus veins, Shaven hairs, Grooveless scutellum, and Eyeless.

INTERFERENCE AND THE COEFFICIENT OF COINCIDENCE

Map distances give us information not only about the distances that separate genes but also about the proportions of recombinant and nonrecombinant gametes that will be produced in a cross. For example, knowing that genes *st* and *ss* on chromosome 3 of *D. melanogaster* are separated by a distance of 14.6 m.u. tells us that 14.6% of the gametes produced by a fly heterozygous at these two loci will be recombinants. Similarly, 12.2% of the gametes from a fly heterozygous for *ss* and *e* will be recombinants.

Theoretically, we should be able to calculate the proportion of double-recombinant gametes by using the multiplication rule (see Chapter 3). Applying this rule, we should find that the proportion (probability) of gametes with double crossovers between *st* and *e* is equal to the probability of recombination between *st* and *ss* multiplied by the probability of recombination between *ss* and *e*, or 0.146 × 0.122 = 0.0178. Multiplying this probability by the total number of progeny gives us the *expected* number of double-crossover progeny from the cross: 0.0178 × 755 = 13.4. But only 8 double crossovers—considerably fewer than the 13 expected—were observed in the progeny of the cross (see Figure 7.14).

This phenomenon is common in eukaryotic organisms. The calculation assumes that each crossover event is independent and that the occurrence of one crossover does not influence the occurrence of another. But crossovers are frequently *not* independent events: the occurrence of one crossover tends to inhibit additional crossovers in the same region of the chromosome, so double crossovers are less frequent than expected.

The degree to which one crossover interferes with additional crossovers in the same region is termed the **interference**. To calculate the interference, we first determine the **coefficient of coincidence**, which is the ratio of observed double crossovers to expected double crossovers:

$$\text{coefficient of coincidence} = \frac{\text{number of observed double crossovers}}{\text{number of expected double crossovers}}$$

For the loci that we mapped on chromosome 3 of *D. melanogaster* (see Figure 7.14), we find that

$$\text{coefficient of coincidence} = \frac{5 + 3}{0.146 \times 0.122 \times 755} = \frac{8}{13.4} = 0.6$$

which indicates that we are actually observing only 60% of the double crossovers that we expected on the basis of the single-crossover frequencies. The interference is calculated as

$$\text{interference} = 1 - \text{coefficient of coincidence}$$

So the interference for our three-point cross is:

$$\text{interference} = 1 - 0.6 = 0.4$$

This interference value tells us that 40% of the double-crossover progeny expected will not be observed because of interference. When interference is complete and no double-crossover progeny are observed, the coefficient of coincidence is 0 and the interference is 1.

Sometimes a crossover *increases* the probability of another crossover taking place nearby, and we see *more* double-crossover progeny than expected. In this case, the coefficient of coincidence is greater than 1 and the interference is negative.

Most eukaryotic organisms exhibit interference, which causes crossovers to be more widely spaced than would be expected on a random basis. Interference was first observed in crosses of *Drosophila* in the early 1900s, yet despite years of study, the mechanism by which

interference occurs is still not well understood. One proposed model of interference suggests that crossovers occur when stress builds up along the chromosome. According to this model, a crossover releases stress for some distance along the chromosome. Because a crossover relieves the stress that causes crossovers, additional crossovers are less likely to occur in the same area. Recent research shows that an enzyme called topoisomerase II is required for crossover interference. Topoisomerase enzymes function to relieve stress along the chromosome, such as torsional stress that occurs during the unwinding of DNA (see Chapters 11 and 12). The fact that a topoisomerase enzyme is required for crossover interference supports the stress release model of crossover interference. **› TRY PROBLEM 31**

THINK-PAIR-SHARE Questions 5 and 6

CONCEPTS

The coefficient of coincidence equals the number of double crossovers observed divided by the number of double crossovers expected on the basis of single-crossover frequencies. The interference equals 1 minus the coefficient of coincidence; it indicates the degree to which one crossover interferes with additional crossovers.

✔ CONCEPT CHECK 6

In analyzing the results of a three-point testcross, a student determines that the interference is −0.23. What does this negative interference value indicate?

a. Fewer double crossovers took place than expected on the basis of single-crossover frequencies.

b. More double crossovers took place than expected on the basis of single-crossover frequencies.
c. Fewer single crossovers took place than expected.
d. A crossover in one region interferes with additional crossovers in the same region.

CONNECTING CONCEPTS

Stepping Through the Three-Point Cross

We have now examined the three-point cross in considerable detail, and we have seen how the information derived from it can be used to map a series of three linked genes. Let's briefly review the steps required to map genes using a three-point cross.

1. **Write out the phenotypes and numbers of progeny produced by the three-point cross.** The progeny phenotypes will be easier to interpret if you use allelic symbols for the traits (such as $st^+ e^+ ss$).
2. **Write out the genotypes of the original parents used to produce the triply heterozygous F_1 individual in the testcross and, if known, the arrangement (coupling or repulsion) of the alleles on their chromosomes.**
3. **Determine which phenotypic classes among the progeny of the testcross are the nonrecombinants and which are the double crossovers.** The nonrecombinants will be the two most common phenotypes; the double crossovers will be the two least common phenotypes.
4. **Determine which locus lies in the middle.** Compare the alleles present in the double crossovers with those present in the nonrecombinants; each class of double crossovers should be like one of the nonrecombinants at two loci and should differ at one locus. The locus that differs is the middle one.
5. **Rewrite the phenotypes with the genes in correct order.**
6. **Determine where crossovers must have taken place to give rise to the recombinant progeny phenotypes.** To do so, compare each phenotype with the phenotype of the nonrecombinant progeny.
7. **Determine the recombination frequencies.** Add the numbers of the progeny that possess a chromosome with a single crossover between a pair of loci. Add the double crossovers to this number. Divide this sum by the total number of progeny from the cross, and multiply by 100%; the result is the recombination frequency between the loci, which is the same as the map distance.
8. **Draw a map of the three loci.** Indicate which locus lies in the middle, and indicate the distances between them.

9. **Determine the coefficient of coincidence and the interference.** The coefficient of coincidence is the number of observed double-crossover progeny divided by the number of expected double-crossover progeny. The expected number can be obtained by multiplying the product of the two single-recombination probabilities by the total number of progeny from the cross.

WORKED PROBLEM

In *D. melanogaster*, cherub wings (*ch*), black body (*b*), and cinnabar eyes (*cn*) result from recessive alleles that are all located on chromosome 2. A homozygous wild-type fly was mated with a cherub, black, cinnabar-eyed fly, and the resulting F_1 females were test-crossed with cherub, black, cinnabar-eyed males. The following progeny were produced by the testcross:

ch	b^+	*cn*	105
ch^+	b^+	cn^+	750
ch^+	*b*	*cn*	40
ch^+	b^+	*cn*	4
ch	*b*	*cn*	753
ch	b^+	cn^+	41
ch^+	*b*	cn^+	102

ch	*b*	cn^+	5
Total			1800

a. Determine the linear order of the genes on the chromosome (which gene is in the middle?).
b. Calculate the map distances between the three loci.
c. Determine the coefficient of coincidence and the interference for these three loci.

Solution Strategy

What information is required in your answer to the problem?

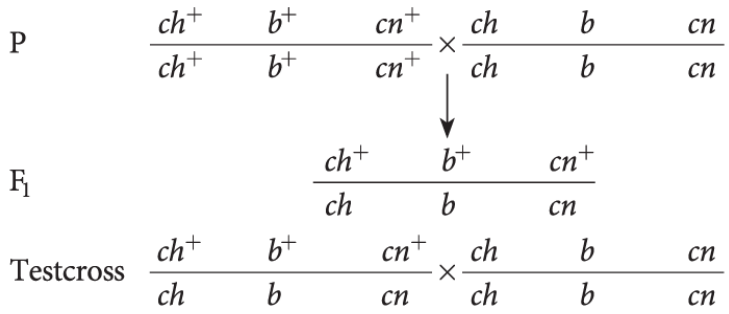
The order of the genes on the chromosome, the map distances among the genes, the coefficient of coincidence, and the interference.

What information is provided to solve the problem?

- A homozygous wild-type fly was mated with a cherub, black, cinnabar-eyed fly, and the resulting F_1 females were test-crossed with cherub, black, cinnabar-eyed males.
- The numbers of the different types of flies appearing among the progeny of the testcross.

Solution Steps

a. We can represent the crosses in this problem as follows:



Description
P generation at the top shows a parent with small C small H superscript positive, small B, and small C small N superscript positive alleles on both the chromosomes crossed with a parent with small C small H, small B, and small C small N on both the chromosomes. Fertilization occurs and F subscript 1 generation is obtained below. The F subscript 1 generation has small C small H superscript positive, small B, and small C small N superscript positive on the top chromosome and small C small H, small B, small C small N on the bottom chromosome. A test cross is done below between a parent with small C small H superscript positive, small B, and small C small N superscript positive alleles on the top chromosome and small C small H, small B, and small C small N on the bottom chromosome and a parent with small C small H, small B, and small C small N on both the chromosomes.

Note that at this point we do not know the order of the genes; we have arbitrarily put *b* in the middle.

The next step is to determine which of the testcross progeny are nonrecombinants and which are double crossovers. The nonrecombinants should be the most frequent phenotype, so they must be the progeny with phenotypes encoded by $\underline{ch^+ \ b^+ \ cn^+}$ and $\underline{\text{ch} \ \ b \ \ cn}$. These genotypes are consistent with the genotypes of the parents, given earlier. The double crossovers are the least frequent phenotypes and are encoded by $\underline{ch^+ \ b^+ \ cn}$ and $\underline{ch \ b \ cn^+}$.

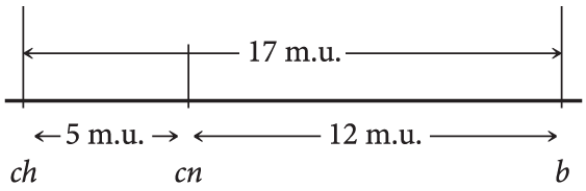
We can determine the gene order by comparing the alleles present in the double crossovers with those present in the nonrecombinants. The double-crossover progeny should be like one of the nonrecombinants at two loci and unlike it at one locus; the allele that differs should be in the middle. Compare the double-crossover progeny $\underline{ch\ b\ cn}^{+}$ with the nonrecombinant $\underline{\text{ch}\quad b\quad cn}$. Both have cherub wings (*ch*) and black body (*b*), but the double-crossover progeny have wild-type eyes (cn^{+}), whereas the nonrecombinants have cinnabar eyes (*cn*). The locus that determines cinnabar eyes must be in the middle.

b. To calculate the recombination frequencies among the genes, we first write the phenotypes of the progeny, with the genes encoding them, in the correct order. We have already identified the nonrecombinant and double-crossover progeny, so the other four progeny types must have resulted from single crossovers. To determine *where* single crossovers took place, we compare the alleles found in the single-crossover progeny with those in the nonrecombinants. Crossing over must have taken place where the alleles switch from those found in one nonrecombinant to those found in the other nonrecombinant. The locations of the crossovers are indicated with a slash:

ch		*cn*	/	b^{+}	105	single crossover
ch^{+}		cn^{+}		b^{+}	750	nonrecombinant
ch^{+}	/	*cn*		*b*	40	single crossover
ch^{+}	/	*cn*	/	b^{+}	4	double crossover
ch		*cn*		*b*	753	nonrecombinant
ch	/	cn^{+}		b^{+}	41	single crossover

ch^+		cn^+	/	b	102	single crossover
ch	/	cn^+	/	b	5	double crossover
Total					1800	

Next, we determine the recombination frequencies and draw a genetic map:



Description
The line drawing of a map shows small C small H gene on the extreme left and 5 m u to its right is gene small C small N, and 12 m u to its right is gene small B. The distance between gene small C small H and gene small B is 17 m u.

$$ch - cn \text{ recombination frequency} = \frac{40 + 4 + 41 + 5}{1800} \times 100\%$$

$$cn\text{-b recombination frequency} = \frac{105 + 4 + 102 + 5}{1800} \times 100\% = 12\%$$

$$ch - b \text{map distance} = 5\% + 12\% = 17\%$$

c. The coefficient of coincidence is the number of observed double crossovers divided by the number of expected double crossovers. The number of expected double crossovers is obtained by multiplying the probability of a crossover between *ch* and *cn* (0.05) by the probability of a crossover between *cn* and *b* (0.12),

then multiplying the product by the total number of progeny from the cross (1800):

$$\text{coefficient of coincidence} = \frac{4+5}{0.05 \times 0.12 \times 1800} = 0.83$$

Finally, the interference is equal to 1− the coefficient of coincidence:

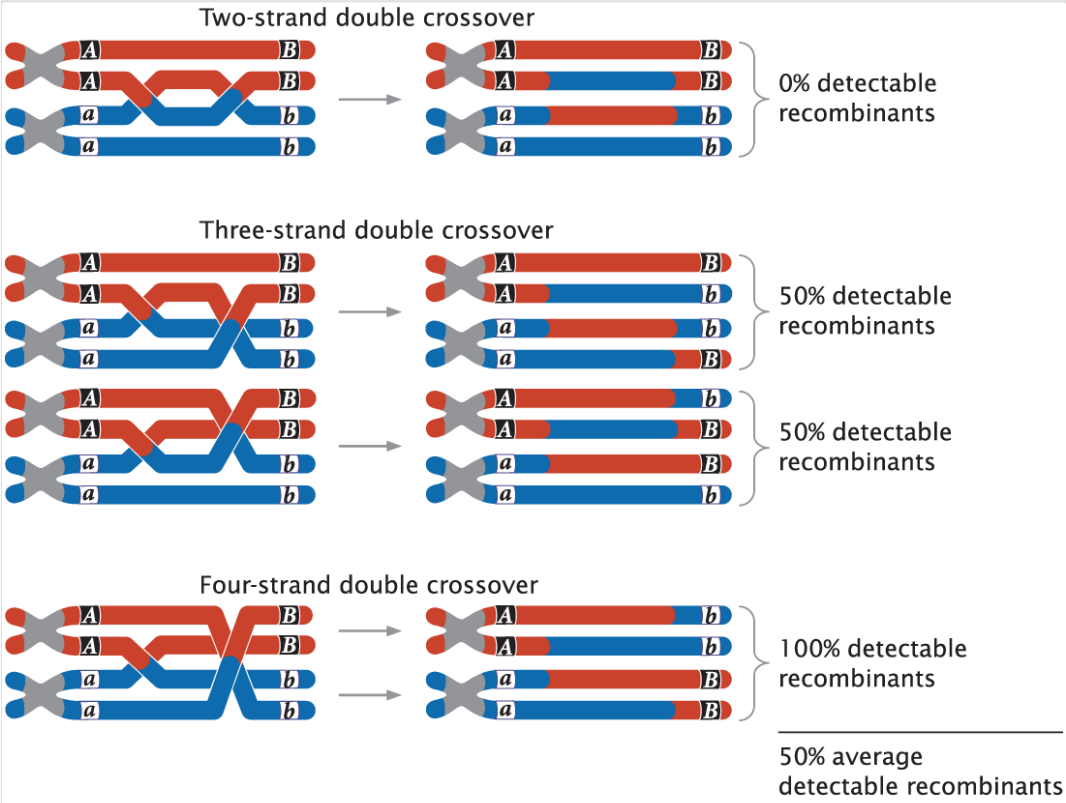
$$\text{interference} = 1 - 0.83 = 0.17$$

» To increase your skill with three-point crosses, try working **Problem 32** at the end of this chapter.

Effects of Multiple Crossovers

So far, we have examined the effects of double crossovers taking place between only two of the four chromatids (strands) of a homologous pair (two-strand double crossovers). Double crossovers that include three and even four of the chromatids of a homologous pair may also take place (**Figure 7.16**). As we have seen, if we examine only the alleles at loci on either side of both crossover events, two-strand double crossovers result in no new combinations of alleles, and no recombinant gametes are produced (see Figure 7.16). Three-strand

double crossovers result in two of the four gametes being recombinant, and four-strand double crossovers result in all four gametes being recombinant (see Figure 7.16). Thus, two-strand double crossovers produce 0% recombination, three-strand double crossovers produce 50% recombination, and four-strand double crossovers produce 100% recombination. The overall result is that all types of double crossovers, taken together, produce an average of 50% recombinant gametes.



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7.16 Effects of two-, three-, and four-strand double crossovers on recombination between two genes.

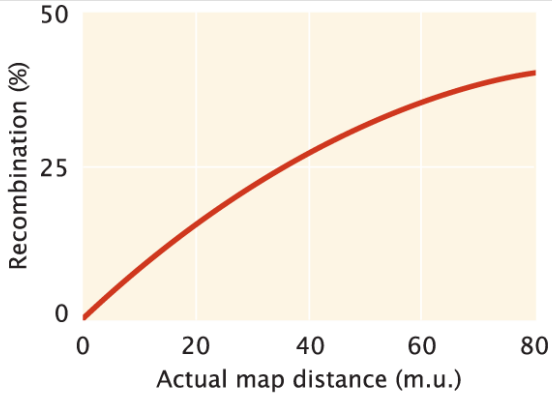
Description

The parent chromosome has red with big A big B on both chromatids and blue with small A and small B on both chromatids. The first part shows a two-strand double crossover where the chromosomes are crossing over at 2 points between the genes. In the progeny chromosomes, one arm of each chromosome is hybrid in color but the genes are not rearranged. A text reads, 0 percent detectable recombinants. The second part below shows a three-strand double crossover occurring and shows 2 examples. The first three-strand crossover shows a red chromosome where the bottom chromosome has blue part with small B allele. The second chromosome is a blue with a red part in between and again a blue part with small B, its bottom chromosome has a red part at the end with big B. A text reads, 50 percent detectable recombinants. The second example shows the first red chromosome with a blue part at the end with small B. Its bottom chromosome has a blue part in between but has same genes as in parent. The blue chromosome has a large red part of top chromosome with big B. The bottom chromosome has same genes as in the parent chromosome. A text reads, 50 percent detectable recombinants. The last part below shows a four-strand double crossover. In the resultant first red chromosome, the top chromosome has a small blue part at the end with small B. The bottom chromosome has a larger blue part with small B. The blue chromosome has large red part with big B and bottom chromosome has a small red part with big B at the end. A text reads, 100 percent detectable Recombinants.

A text below reads, 50 percent average detectable recombinants.

As we have also seen, two-strand double crossovers cause alleles on either side of the crossovers to remain the same and produce no recombinant progeny. Three-strand and four-strand crossovers produce recombinant progeny, but these progeny are the same types as those produced by single crossovers. Consequently, some multiple crossovers go undetected when the progeny of a genetic cross are observed. Therefore, map distances based on recombination rates underestimate the true physical distances between genes because some multiple crossovers are not detected among the progeny of a cross. When genes are very close together, multiple crossovers are

unlikely, and genetic distances based on recombination rates accurately correspond to the physical distances on the chromosome. But as the distance between genes increases, more multiple crossovers are likely, and the discrepancy between genetic distances (based on recombination rates) and physical distances increases. To correct for this discrepancy, geneticists have developed mathematical **mapping functions**, which relate recombination frequencies to actual physical distances between genes (**Figure 7.17**). Most of these functions are based on the Poisson distribution, which predicts the probability of multiple rare events. With the use of such mapping functions, map distances based on recombination rates can be more accurately estimated.



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7.17 Recombination rates underestimate the true physical distance between genes at higher map distances.

Description

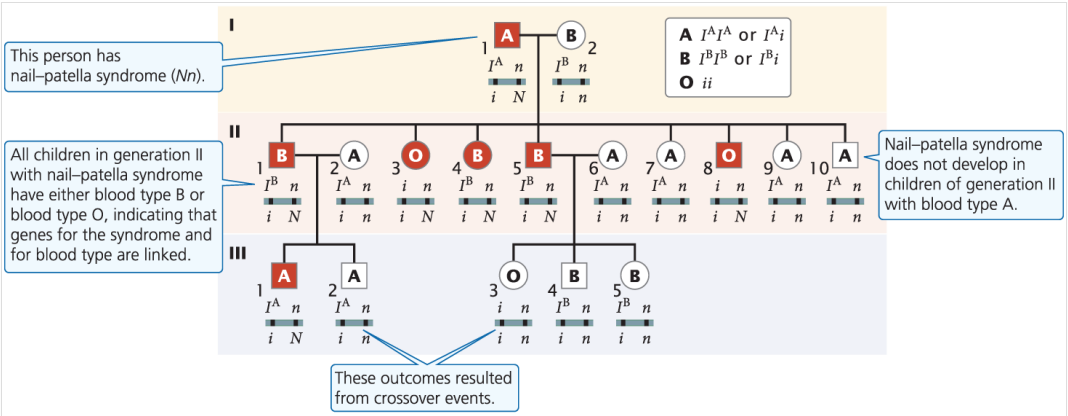
The horizontal axis is labeled as the actual map distance in m u from 0 to 80 with an interval of 20. The vertical axis shows recombination frequency from 0 to 50 percent with an interval of 25. The approximate data from the graph are as follows: the curve begins at the origin and

rises up to reach 27 percent recombination at 40 m u, and it eventually reaches 40 percent at 80 m u.

Mapping Human Genes

Efforts to map human genes are hampered by the inability to perform specific crosses and by the small number of progeny in most human families. Geneticists are often restricted to analyses of pedigrees, which may be incomplete and may provide limited information. Nevertheless, a large number of human traits have been successfully mapped with the use of pedigree data to analyze linkage. Because the number of progeny from any one mating is usually small, data from several families and pedigrees are usually combined to test for independent assortment. The methods used in these types of analyses are complex, but an example will illustrate how linkage can be detected from pedigree data.

One of the first documented demonstrations of linkage in humans was between the locus for nail–patella syndrome and the locus that determines the ABO blood types. Nail–patella syndrome is an autosomal dominant disorder characterized by abnormal fingernails and absent or rudimentary kneecaps. The ABO blood types are determined by an autosomal locus with multiple alleles (see Chapter 5). Linkage between the genes encoding these two traits was established in families in which both traits segregate. A pedigree for part of one such family is illustrated in **Figure 7.18**.



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7.18 Linkage between ABO blood type and nail–patella syndrome was established by examining families in which both traits segregate. The pedigree shown here is for one such family. The ABO blood type is indicated in each circle or square. The genotype, inferred from the phenotype, is given below each circle or square.

Description

Generation 1 shows parents where affected male has blood group A (big I superscript A small N small I big N). A caption reads, This person has nail–patella syndrome (big N small N). In same generation, an unaffected female has blood group B (big I superscript B small N small I small N). They mate and have 3 affected sons, 2 affected daughters, 2 unaffected daughters, and 1 unaffected son in generation 2. A caption reads, All children in generation 2 with nail–patella syndrome have either blood type B or blood type O, indicating that genes for the syndrome and for blood type are linked. The following chromosomes are obtained: First affected son has B blood group (big I superscript B small I big N). First affected daughter has O blood group (small I small N small I big N). The second affected daughter has B blood group (big I superscript B small N small I big N). The second affected son has B blood group (big I superscript B small N small I big N). The first unaffected daughter has A blood group (big I superscript A small N small I small N). The third affected son has O blood group (small I small N small I big N). The second unaffected daughter has A blood group (big I superscript A small N small I small N). The unaffected son has A blood group (big I superscript A small N small I small N). A caption reads, Nail–patella syndrome does not develop in children of generation 2 with blood type A. The first affected male from generation 2 who has B blood group (big I superscript B small I big N) mates with an unaffected female with A blood group (big I superscript A small N small I small N) and has an affected son and an unaffected son in generation 3. The affected son has A blood group (big I superscript A small N small I big N) and unaffected son has A blood group (big I superscript A small N small I small N). A caption

reads, These outcomes resulted from crossover events. The second affected male has B blood group (big I superscript B small N small I big N) mates with an unaffected female with blood group A (big I superscript A small N small I small N). They have 2 unaffected daughters and 1 affected son in generation 3. The first unaffected daughter has O blood group (small I small N small I small N). The unaffected son has B blood group (big I superscript B small N small I small N). The second unaffected daughter has B blood group (big I superscript B small N small I small N).

Nail–patella syndrome is rare, so we can assume that people who have this trait are heterozygous (*Nn*); unaffected people are homozygous (*nn*). The ABO genotypes of individuals can be inferred from their phenotypes and the types of offspring they produce. Female I-2 in Figure 7.18, for example, has blood type B, which has two possible genotypes: I^BI^B or I^Bi (see Figure 5.6). Because some of her offspring are blood type O (genotype *ii*) and must have therefore inherited an *i* allele from each parent, female I-2 must have genotype I^Bi. Similarly, the presence of blood type O offspring in generation II indicates that male I-1, with blood type A, must also carry an *i* allele and therefore has genotype I^Ai. The parents of this family are

$$I^Ai\ Nn \times I^Bi\ nn$$

In generation II, we can see that the genes for nail–patella syndrome and the blood types do not appear to assort independently. All children in generation II with nail–patella syndrome have either blood type B or blood type O; all those with blood type A have normal nails and kneecaps. If the genes encoding nail–patella syndrome and the ABO blood types assorted independently, we would expect that some children in generation II would have blood type A and nail–patella

syndrome, inheriting both the I^A and N alleles from their father. The observed outcome indicates that the arrangements of the alleles on the chromosomes of the crossed parents are

$$\frac{I^A \quad n}{i \quad N} \times \frac{I^B \quad n}{i \quad n}$$

Description
Each chromosome is represented by a straight, horizontal line. For first chromosome: the alleles above the line are big I superscript big A small N and alleles below the line are small I big N.

For second chromosome: the alleles above the line are big I superscript big B small N and alleles below the line are small I small N. A cross sign is present between the chromosomes.

The pedigree indicates that there is no recombination among the offspring (generation II) of these parents, but there are two instances of recombination among the offspring in generation III. Individuals II-1 and II-2 have the following genotypes:

$$\frac{I^B \quad n}{i \quad N} \times \frac{I^A \quad n}{i \quad n}$$

Description
Each chromosome is represented by a straight, horizontal line. For first chromosome: the alleles above the line are big I superscript big B small N and alleles below the line are small I big N. For second chromosome: the alleles above the line are big I superscript big A small N and alleles below the line are small I small N. A cross sign is present between the chromosomes.

Their child III-2 has blood type A and does not have nail–patella syndrome, so he must have genotype

$$\frac{I^{A} \quad n}{i \quad n}$$

Description
The chromosome is represented by a straight, horizontal line. The alleles above the line are big I superscript big A small N and alleles below the line are small I small N.

and must have inherited both the *i* and the *n* alleles from his father. These alleles are on different chromosomes in the father, so crossing over must have taken place. Crossing over must also have taken place to produce child III-3.

In the pedigree in Figure 7.18, thirteen children are offspring of matings in which the genes encoding nail–patella syndrome and ABO blood types segregate; two of the thirteen children are recombinant progeny. On this basis, we might assume that the loci for nail–patella syndrome and ABO blood types are linked, with a recombination frequency of $^{2}/_{13} = 0.154$. However, it is possible that the genes *are* assorting independently, and that the small number of children just makes it seem as though the genes are linked.

To determine the probability that genes are actually linked, geneticists often calculate **lod** (logarithm of odds) **scores**. To obtain a lod score, we calculate (1) the probability of obtaining the observed results under the assumption that the genes are linked with a specified degree of recombination and (2) the probability of obtaining

the observed results under the assumption of independent assortment. We then determine the ratio of these two probabilities, and the logarithm of this ratio is the lod score. Suppose that the probability of obtaining a particular set of observations under the assumption of linkage and a certain recombination frequency is 0.1, and that the probability of obtaining the same observations under the assumption of independent assortment is 0.0001. The ratio of these two probabilities is $^{0.1}/_{0.0001} = 1000$, the logarithm of which (the lod score) is 3. Thus, linkage with the specified recombination frequency is 1000 times as likely as independent assortment to produce what was observed. A lod score of 3 or higher is usually considered convincing evidence for linkage. › **TRY PROBLEM 38**

Mapping with Molecular Markers

For many years, gene mapping was limited in most organisms by the availability of **genetic markers**: variable genes with easily observable phenotypes whose inheritance could be studied. Traditional genetic markers include genes that encode easily observable characteristics, such as flower color, seed shape, blood types, or biochemical differences. The paucity of these types of characteristics in many organisms limited mapping efforts.

In the 1980s, new molecular techniques made it possible to examine variations in DNA itself, providing an almost unlimited number of genetic markers. The earliest of these molecular markers consisted of

restriction fragment length polymorphisms (RFLPs), which are variations in DNA sequence that can be detected by cutting the DNA with restriction enzymes. Later, methods were developed for detecting variable numbers of short DNA sequences repeated in tandem, called microsatellites. Now DNA sequencing allows the direct detection of individual variations in the DNA nucleotides. All of these methods have expanded the availability of genetic markers and greatly facilitated the creation of genetic maps.

Gene mapping with molecular markers is done in essentially the same manner as mapping performed with traditional phenotypic markers: the cosegregation of two or more markers is studied, and map distances are based on the rates of recombination between markers. These methods and their use in mapping are presented in more detail in Chapters 19 and 20.

Locating Genes with Genome-Wide Association Studies

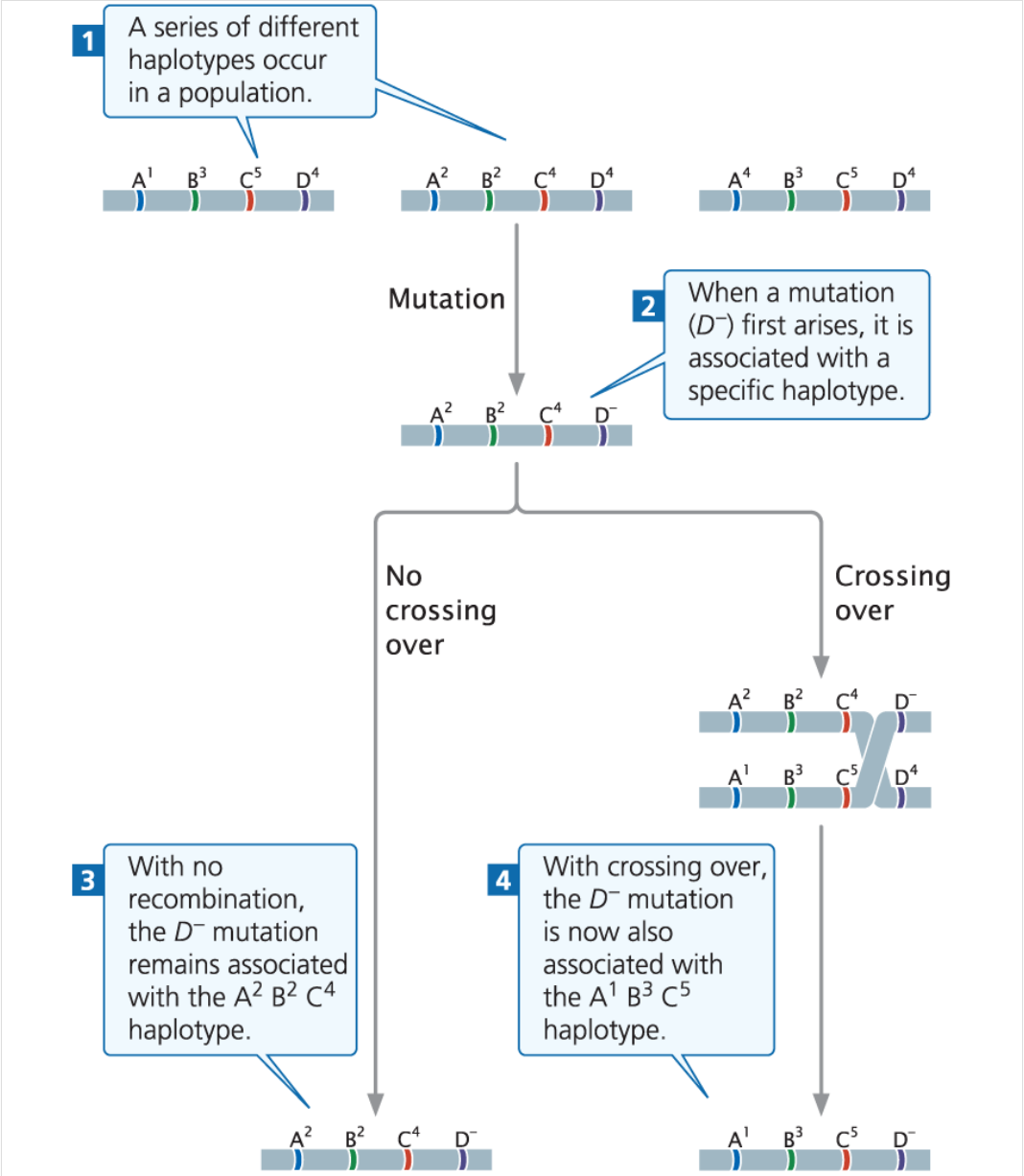
The traditional approach to mapping genes, which we have learned in this chapter, is to examine progeny phenotypes in genetic crosses or among individuals in a pedigree, looking for associations between the inheritance of a particular phenotype and the inheritance of alleles at other loci. This type of gene mapping is called **linkage analysis** because it is based on the detection of physical linkage between genes, as measured by the rate of recombination, in the progeny of a

cross. Linkage analysis has been a powerful tool in the genetic analysis of many different types of organisms, including fruit flies, corn, mice, and humans.

An alternative approach to mapping genes is to conduct **genome-wide association studies**, looking for nonrandom associations between the presence of a trait and alleles at many different loci scattered across the genome. Unlike linkage analysis, this approach does not trace the inheritance of genetic markers and traits in a genetic cross or family. Rather, it looks for associations between traits and particular suites of alleles in a *population*.

Imagine that we are interested in finding genes that contribute to bipolar disorder, a behavioral illness characterized by severe depression and mania. When a mutation that predisposes a person to bipolar disorder first arises in a population, it will occur on a particular chromosome and will be associated with a specific set of alleles on that chromosome. In the example illustrated in **Figure 7.19**, the D^- mutation first arises on a chromosome that has alleles A^2, B^2, and C^4, and therefore the D^- mutation is initially linked to the A^2, B^2, and C^4 alleles. A specific set of linked alleles such as this is called a **haplotype**, and the nonrandom association between alleles in a haplotype is called **linkage disequilibrium**. Because of the physical linkage between the bipolar mutation and the other alleles of the haplotype, bipolar disorder and the haplotype will tend to be inherited together. Crossing over, however, breaks up the association between the alleles of the haplotype (see Figure 7.19), reducing the linkage

disequilibrium between them. How long the linkage disequilibrium persists over evolutionary time depends on the frequency of recombination between alleles at different loci. When the loci are far apart, linkage disequilibrium breaks down quickly; when the loci are close together, crossing over is less common and linkage disequilibrium persists longer. The important point here is that linkage disequilibrium provides information about the distance between genes. A strong association between a trait such as bipolar disorder and a set of linked genetic markers indicates that one or more genes contributing to bipolar disorder are likely to be near the genetic markers.



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7.19 Genome-wide association studies are based on the nonrandom association of a mutation (D^-) that produces a trait with closely linked genes that constitute a haplotype.

Description

The diagram shows 3 different chromosomes. The first chromosome has the following alleles: A superscript 1, B superscript 3, C superscript 5, and D superscript 4. A caption

reads, A series of different haplotypes occur in a population. The second chromosome has following alleles: A superscript 2, B superscript 2, C superscript 4, and D superscript 4. The third chromosome has following alleles: A superscript 4, B superscript 3, C superscript 5, and D superscript 4. Mutation occurs in the chromosome 2. The D superscript 4 allele changes to D superscript negative. A caption reads, When a mutation (D superscript negative) first arises, it is associated with a specific haplotype. No crossing over occurs and the chromosome has same alleles. A caption reads, With no recombination, the D negative mutation remains associated with the A superscript 2 B superscript 2 C superscript 4 haplotype. Crossing over occurs between the mutated chromosome (A superscript 2, B superscript 2, C superscript 4, and D superscript negative) and a chromosome (A superscript 1, B superscript 3, C superscript 5, and D superscript 4). The resultant chromosome below is A superscript 1 B superscript 3 C superscript 5, and D superscript negative. A caption reads, With crossing over, the D negative mutation is now also associated with the A superscript 1 B superscript 3 C superscript 5 haplotype.

In recent years, geneticists have mapped millions of **single-nucleotide polymorphisms (SNPs)**, which are positions in the genome where individuals vary in a single nucleotide base (see the introduction to this chapter and Chapter 20). Recall that human SNPs were used to map genes associated with fear and aggression in dogs, as discussed in the introduction to this chapter. It is now possible to quickly and inexpensively genotype people for hundreds of thousands or millions of SNPs. This genotyping has provided the genetic markers needed for conducting genome-wide association studies in which SNP haplotypes of people who have a particular disease, such as bipolar disorder, are compared with the haplotypes of healthy people. Nonrandom associations between SNPs and the disease suggest that one or more genes that contribute to the disease are closely linked to the SNPs. Genome-wide association studies do not usually locate specific genes: rather, they associate the inheritance of a trait or

disease with a specific chromosomal region. After such an association has been established, geneticists can examine the chromosomal region for genes that might be responsible for the trait. Genome-wide association studies have been instrumental in the discovery of genes or chromosomal regions that affect a number of genetic diseases and important human traits, including bipolar disorder, height, skin pigmentation, eye color, body weight, coronary artery disease, blood-lipid concentrations, diabetes, heart attacks, bone density, and glaucoma. Genome-wide association studies are also being used to locate and study genes that affect traits in numerous other organisms.

THINK-PAIR-SHARE Question 7

CONCEPTS

The development of molecular techniques for examining variation in DNA sequences has provided a large number of genetic markers that can be used to create genetic maps and study linkage relations. Genome-wide association studies examine the nonrandom association of genetic markers and phenotypes to locate genes that contribute to the expression of traits.

7.4 Physical-Mapping Methods Are Used to Determine the Physical Positions of Genes on Particular Chromosomes

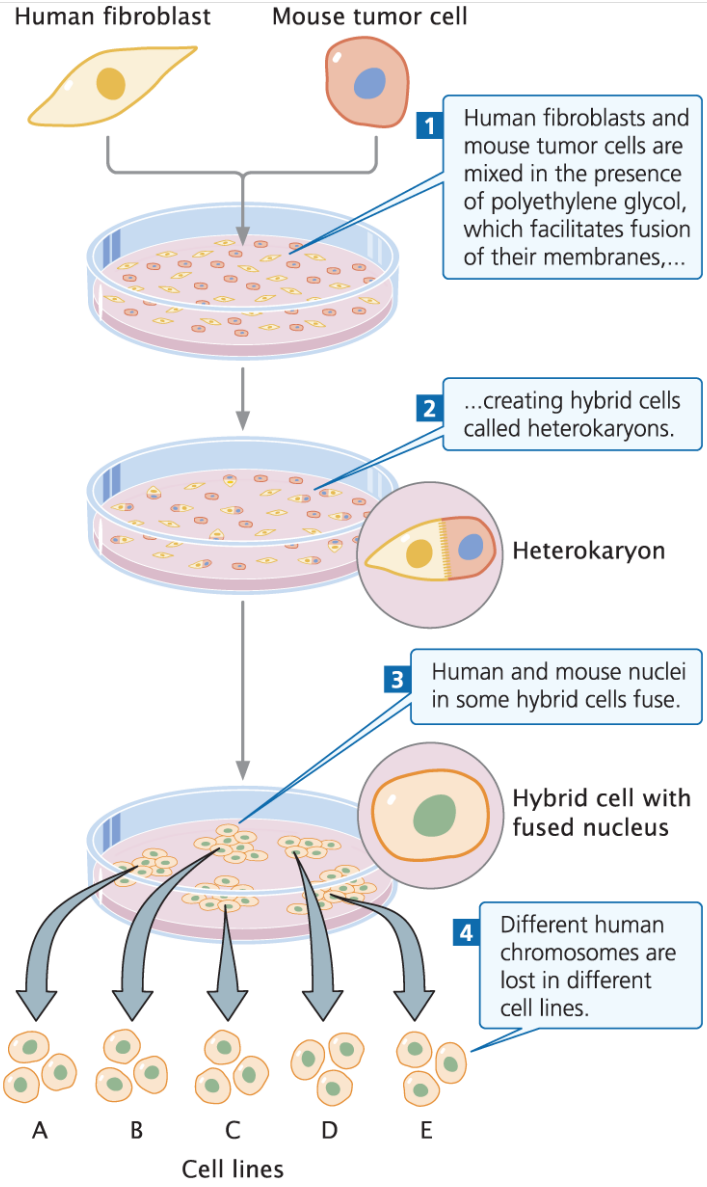
Genetic maps reveal the relative positions of genes on a chromosome, but they do not provide information that allows us to place groups of linked genes on particular chromosomes. Furthermore, the units of a genetic map do not always precisely correspond to physical distances on the chromosome, because a number of factors other than physical distances between genes (such as the type and sex of the organism) can influence recombination. Because of these limitations, physical-mapping methods that do not rely on recombination frequencies have been developed.

Somatic-Cell Hybridization

One method used for positioning genes on chromosomes is **somatic-cell hybridization**, which requires the fusion of different types of cells. Most mature somatic (nonsex) cells can undergo only a limited number of divisions and therefore cannot be grown continuously. However, cells that have been altered by viruses or derived from tumors that have lost the normal constraints on cell division will

divide indefinitely; this type of cell can be cultured in the laboratory to produce a **cell line**.

Cells from two different cell lines can be fused by treating them with polyethylene glycol or other agents that alter their plasma membranes. The resulting cell possesses two nuclei and is called a **heterokaryon**. The two nuclei of a heterokaryon eventually also fuse, generating a hybrid cell that contains chromosomes from both cell lines. If human and mouse cells are mixed in the presence of polyethylene glycol, their fusion results in human–mouse somatic-cell hybrids (**Figure 7.20**). The hybrid cells tend to lose chromosomes as they divide, and for reasons that are not understood, chromosomes from one of the species are lost preferentially. In human–mouse somatic-cell hybrids, the human chromosomes tend to be lost, whereas the mouse chromosomes are retained. Eventually, the chromosome number stabilizes when all but a few of the human chromosomes have been lost. Chromosome loss is random and differs among cell lines. The presence of these "extra" human chromosomes in the mouse genome makes it possible to assign human genes to specific chromosomes.



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7.20 Somatic-cell hybridization can be used to determine which chromosome contains a gene of interest.

Description

A spindle-shaped human fibroblast and a round mouse tumor cell fuse in a petri dish below to give hybrid cells. A caption reads, Human fibroblasts and mouse tumor cells are mixed in the presence of polyethylene glycol, which facilitates fusion of their membranes creating hybrid cells called heterokaryons. The resultant hybrid cells have fused membrane with

two distinct nuclei. This leads to the formation of hybrid cells with fused nucleus. Five clusters of cells are obtained below. A caption reads, Human and mouse nuclei in some hybrid cells fuse. These clusters are marked as different cell lines named A, B, C, D, and E. A caption reads, Different human chromosomes are lost in different cell lines.

Mapping genes by using somatic-cell hybridization requires a panel of different hybrid cell lines. Each cell line is examined microscopically, and the human chromosomes that it contains are identified. The cell lines of the panel are chosen so that they differ in the human chromosomes that they have retained. For example, one hybrid cell line might possess human chromosomes 2, 4, 7, and 8, whereas another might possess chromosomes 4, 19, and 20. Each cell line in the panel is examined for evidence of a particular human gene. The human gene can be detected by looking either for the gene itself (as discussed in Chapter 19) or for the protein that it produces. Correlation of the presence of the gene with the presence of specific human chromosomes often allows the gene to be assigned to the correct chromosome. For example, if a gene is detected in both of the aforementioned cell lines, the gene must be on chromosome 4 because it is the only human chromosome common to both cell lines (**Figure 7.21**). Somatic-cell hybridization is used less often today because DNA sequencing and mapping with molecular markers can often establish the chromosomal locations of linkage groups.

		Human chromosomes present																						
Cell line	Gene product present	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
A	+		+		+			+	+															
B	+	+	+		+				+	+	+	+	+	+										
C	–															+		+		+			+	
D	+		+		+		+	+	+															
E	–												+								+			
F	+				+															+	+			

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7.21 Somatic-cell hybridization is used to assign a gene to a particular human chromosome. A panel of six hybrid cell lines, each containing a different subset of human chromosomes, is examined for the presence of the gene product (such as an enzyme). Four of the cell lines (A, B, D, and F) express the gene product. The only chromosome common to all four of these cell lines is chromosome 4, indicating that the gene is located on this chromosome.

Description

The column headers are as follows: cell line, gene product present, and the numbers 1 to 22, and X.

The row entries are as follows:

Row 1: A; gene product present, positive; then columns numbered 2, 4, 7, and 8 show positive.

Row 2: B; gene product present, positive; then columns numbered 1, 2, 4, and 8 to 13 show positive.

Row 3: C; gene product present, negative; then columns numbered 15, 17, 19, and 22 show positive.

Row 4: D; gene product present, positive; then columns numbered 2, 4, and 6 to 8 show positive.

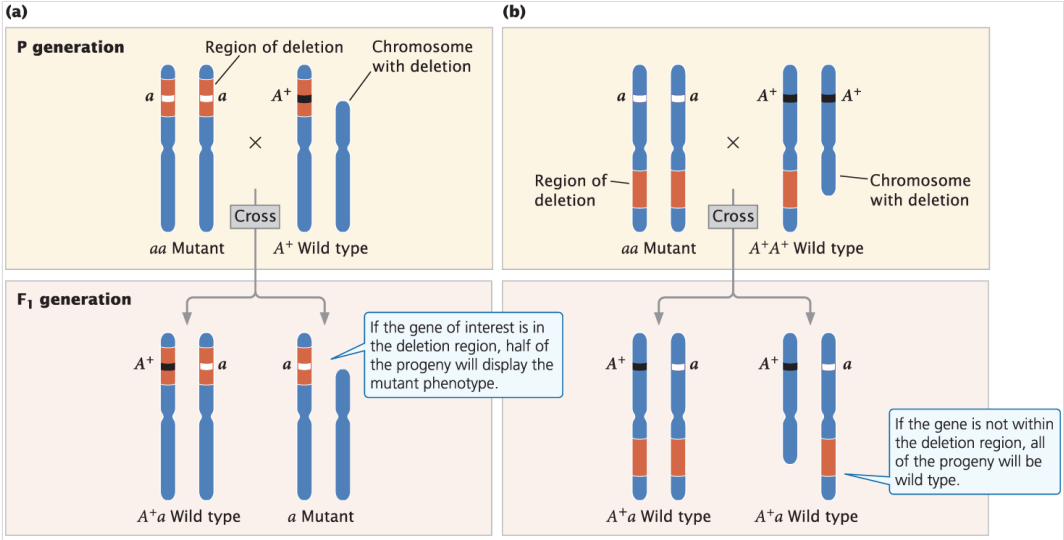
Row 5: E; gene product present, negative; then columns numbered 12 and 20 show positive.

Row 6: F; gene product present, positive; then columns numbered 4, 19, and 20 show positive.

Deletion Mapping

Another method for determining the chromosomal location of a gene is **deletion mapping**. Special staining methods have been developed that reveal characteristic banding patterns on chromosomes (see Chapter 8). The absence of one or more of the bands that are normally seen on a chromosome reveals the presence of a chromosomal *deletion* (loss of a chromosome segment). We can assign genes to regions of chromosomes by studying the association between a phenotype or product encoded by a gene and particular chromosomal deletions.

In deletion mapping, an individual that is homozygous for a recessive mutation in the gene of interest is crossed with an individual that is heterozygous for a deletion (**Figure 7.22**). If the gene of interest is in the region of the chromosome represented by the deletion (the red part of the chromosomes in Figure 7.22), then approximately half of the progeny will display the mutant phenotype (see Figure 7.22a). If the gene is not within the deleted region, then all of the progeny will be wild type (see Figure 7.22b).



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7.22 Deletion mapping can be used to determine the chromosomal location of a gene. An individual homozygous for a recessive mutation in the gene of interest (*aa*) is crossed with an individual heterozygous for a deletion.

Description

Part a on the left shows P generation at the top where a cross between an individual with genotype small A small A (small A small A mutant) and an individual with genotype big A superscript positive and a chromosome with deletion is done (big A superscript positive wild type). These chromosomes are crossed and the F subscript 1 generation obtained below has two progenies: a heterozygote with big A superscript positive on one chromosome and small A on other (big A superscript positive small A wild type), and a mutant progeny with genotype small A on one chromosome. A caption reads, If the gene of interest is in the deletion region, half of the progeny will display the mutant phenotype. Part b on the right shows P generation at the top. An individual has genotype homozygous small A on one chromosome and small A on other. The region of deletion is present on the both the chromosome below the centromere (small A small A mutant). Another individual has big A superscript positive on both the chromosomes above the centromere and has second chromosome has a deletion (big A superscript positive big A superscript positive wild type). These 2 individuals cross and yield an F subscript 1 generation below. The big A superscript positive small A wild type progeny has big A superscript positive allele on the first chromosome and small A on the other chromosome. Both chromosomes have a region of deletion below respective centromeres. Another big A superscript positive small A wild type progeny has first chromosome with big A superscript positive and short arm

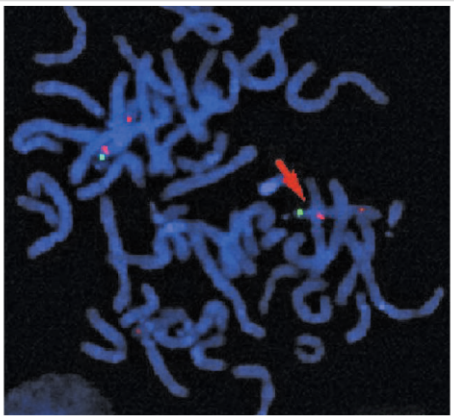
below centromere and second chromosome has small A with a region of deletion below its centromere. A caption reads, If the gene is not within the deletion region all of the progeny will be wild type.

Deletion mapping has been used in the past to reveal the chromosomal locations of a number of human genes. For example, Duchenne muscular dystrophy is a disease that causes progressive weakening and degeneration of the muscles (see introduction to Chapter 19). From its X-linked pattern of inheritance, the mutant allele causing this disorder was known to be on the X chromosome, but its precise location was uncertain. Examination of a number of patients with the disease who also possessed small deletions allowed researchers to position the gene on a small segment of the short arm of the X chromosome. Like somatic-cell hybridization, deletion mapping is used less often today because of the widespread availability of DNA sequencing. › **TRY PROBLEM 41**

Physical Chromosome Mapping Through Molecular Analysis

So far, we have explored methods that determine the chromosomal location of a gene indirectly. Researchers now have the information and technology to see where a gene actually lies. Described in more detail in Chapter 19, fluorescence in situ hybridization (FISH) is a method for determining the chromosomal location of a particular gene through molecular analysis. This method requires the creation

of a probe for the gene, which is a single-stranded DNA complement to the gene of interest. The probe fluoresces under high-frequency light so that it can be visualized. The probe binds to the DNA sequence of the gene of interest on the chromosome. The presence of fluorescence from the bound probe reveals the location of the gene on a particular chromosome (**Figure 7.23**).



Prader Willi & Angelman syndromes - FISH. Wessex Reg. Genetics Centre. CC BY.

7.23 In situ hybridization is another technique for determining the chromosomal location of a gene. The red and green fluorescence spots are produced by probes that are specific to different DNA sequences on chromosome 15.

In addition to allowing us to see where a gene is located on a chromosome, modern laboratory techniques now allow researchers to identify the precise location of a gene at the nucleotide level. For example, with DNA sequencing (described fully in Chapter 19), physical distances between genes can be determined in numbers of base pairs.

CONCEPTS

Physical-mapping methods determine the physical locations of genes on chromosomes. They include somatic-cell hybridization, deletion mapping, in situ hybridization, and direct DNA sequencing.

7.5 Recombination Rates Exhibit Extensive Variation

Geneticists have studied variation in rates of recombination and have found that they vary widely—among species, among and along chromosomes of a single species, and even between males and females of the same species. For example, about twice as much recombination takes place in humans as in mice and rats. Within the human genome, recombination rates vary among chromosomes, with chromosomes 21 and 22 having the highest rates and chromosomes 2 and 4 having the lowest rates. Researchers have also detected differences between male and female humans in rates of recombination: the autosomal chromosomes of females undergo about 50% more recombination than do the autosomal chromosomes of males.

Geneticists have found numerous recombination *hotspots*, where recombination is at least 10 times as high as the average elsewhere in the genome. The human genome contains an estimated 25,000 to 50,000 such recombination hotspots, and approximately 60% of all crossovers take place in them. For humans, recombination hotspots tend to be found near, but not within, active genes. Recombination hotspots have been detected in the genomes of other organisms as well. Other chromosomal regions, such as those near centromeres, often display reduced rates of recombination.

CONCEPTS

Rates of recombination vary among species, among and along chromosomes, and even between males and females.

Chapter 7 Review

CHAPTER SUMMARY

Section 7.1 Learning Objective: Be able to explain how the inheritance of linked genes is different from those that are not linked.

- Linked genes do not assort independently.

Section 7.2 Learning Objective: Be able to analyze crosses involving linked genes.

- In a testcross for two completely linked genes (with no crossing over), only nonrecombinant progeny are produced. When two genes assort independently, recombinant progeny and nonrecombinant progeny are produced in equal proportions. When two genes are linked with some crossing over between them, more nonrecombinant progeny than recombinant progeny are produced.
- Recombination frequency is calculated by summing the number of recombinant progeny, dividing by the total number of progeny produced in the cross, and multiplying by 100%. The frequency of recombinant gametes is half the frequency of crossing over, and the maximum frequency of recombinant gametes is 50%.
- Coupling and repulsion refer to the arrangement of alleles on a chromosome. Whether alleles are in coupling or in repulsion determines which combination of phenotypes will be most frequent in the progeny of a testcross.
- Interchromosomal recombination takes place among genes located on different chromosomes through the random segregation of

chromosomes in meiosis. Intrachromosomal recombination takes place among genes located on the same chromosome through crossing over.
- A chi-square test of independence can be used to determine whether genes are linked.
- Recombination rates can be used to determine the relative order of genes and distances between them on a chromosome. One percent recombination equals one map unit. Maps based on recombination rates are called genetic maps; maps based on physical distances are called physical maps.
- Genetic maps can be constructed by examining recombination rates from a series of two-point crosses.

Section 7.3 Learning Objective: Using data from a three-point testcross, be able to map a series of three linked genes, including determination of the order of the genes, the recombination frequencies, and the interference.

- Genetic maps can be constructed by examining the progeny of a three-point testcross.
- Interference is the degree to which one crossover interferes with additional crossovers in the same area. The interference equals 1 minus the coefficient of coincidence, which is the observed number of double crossovers divided by the expected number of double crossovers.
- Some multiple crossovers go undetected; thus, genetic maps based on recombination rates underestimate the true physical distances between genes.
- Human genes can be mapped by examining the cosegregation of traits in pedigrees.
- A lod score is the logarithm of the ratio of the probability of obtaining the observed progeny with the assumption of linkage to the probability of obtaining the observed progeny with the assumption of independent

assortment. A lod score of 3 or higher is usually considered evidence for linkage.
- Molecular techniques that allow the detection of differences in DNA sequence have greatly facilitated gene mapping.
- Genome-wide association studies locate genes that affect particular traits by examining the nonrandom association of a trait with sets of genetic markers from across the genome.

Section 7.4 Learning Objective: Be able to describe the methods used in physical gene mapping, including somatic cell hybridization, deletion mapping, and fluorescence in situ hybridization (FISH).

- Physical mapping methods are used to determine the chromosomal locations of genes.
- Nucleotide sequencing is another method of physically mapping genes.

Section 7.5 Learning Objective: Be able to describe variation in recombination rates.

- Rates of recombination vary widely, differing among species, among and along chromosomes within a single species, and even between males and females of the same species.

WORKED PROBLEMS

Problem 1

In guinea pigs, white coat (*w*) is recessive to black coat (*W*), and wavy hair (*v*) is recessive to straight hair (*V*). A breeder crosses a guinea pig that is homozygous for white coat and wavy hair with a guinea pig that is black with straight hair. The F_1 are then crossed with guinea pigs having white

coats and wavy hair in a series of testcrosses. The following progeny are produced from these testcrosses:

black, straight	30
black, wavy	10
white, straight	12
white, wavy	31
Total	83

a. Are the genes that determine coat color and hair type assorting independently? Carry out chi-square tests to test your hypothesis.
b. If the genes are not assorting independently, what is the recombination frequency between them?

» Solution Strategy

What information is required in your answer to the problem?

a. Whether the genes are assorting independently, along with a chi-square value, degrees of freedom, and P value to evaluate your hypothesis.
b. If the genes are not assorting independently, the recombination frequency between them.

What information is provided to solve the problem?

- White coat is recessive to black coat, and wavy hair is recessive to straight hair.

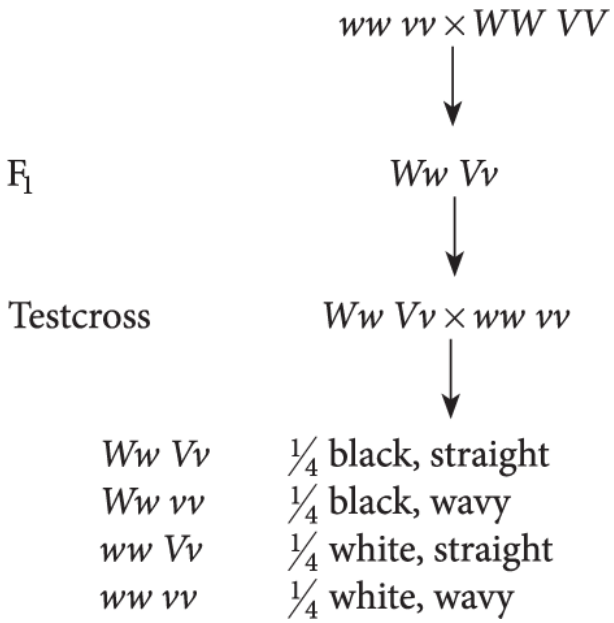
- The numbers of different types of progeny from a series of testcrosses.

For help with this problem, review:

Crossing Over Between Linked Genes, Calculating Recombination Frequency, and Testing for Independent Assortment in Section 7.2.

» Solution Steps

a. Assuming independent assortment, outline the crosses conducted by the breeder:



Description
P generation at the top shows a guinea pig with small W small W small V small V crossed with big W big W big V big V. F subscript 1 generation below shows genotype big W small W big V small V. A test cross is conducted between big W small W big V small V and small W small W small V small V. The genotypes obtained below are:

big W small W big V small V (one-fourth black, straight).

big W small W small V small V (one-fourth black, wavy).

small W small W big V small V (one-fourth white, straight).

small W small W small V small V (one-fourth white, wavy).

A caption reads, Recall: With independent assortment, we expect equal numbers of nonrecombinant and recombinant offspring.

Recall: With independent assortment, we expect equal numbers of nonrecombinant and recombinant offspring.

Because a total of 83 progeny were produced in the testcrosses, we expect $^1/_4 \times 83 = 20.75$ of each. The observed numbers of progeny from the testcross (30, 10, 12, 31) do not appear to fit the expected numbers (20.75, 20.75, 20.75, 20.75) well, so independent assortment may not have taken place.

Hint: See Figure 7.10 for a review of how to carry out a chi-square test of independence.

To test the hypothesis, carry out a chi-square test of independence. Construct a table with the genotypes of the first locus along the top and the genotypes of the second locus along the side. Compute the totals for the rows and columns and the grand total.

	Ww	*ww*	Row totals
Vv	30	12	42
vv	10	31	41
Column totals	40	43	83 ← Grand total

The expected value for each cell of the table is calculated with the following formula:

$$\text{expected number} = \frac{\text{row total} \times \text{column total}}{\text{grand total}}$$

Using this formula, we find the expected values (given in parentheses) to be

	Ww	*ww*	Row totals
Vv	30 (20.24)	12 (21.76)	42
vv	10 (19.76)	31 (21.24)	41
Column totals	40	43	83 ← Grand total

Using these observed and expected numbers, we find the calculated chi-square value to be

$$\begin{aligned}
\chi^2 &= \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}} \\
&= \frac{(30 - 20.24)^2}{20.24} + \frac{(10 - 19.76)^2}{19.76} + \frac{(12 - 21.76)^2}{21.76} + \frac{(31 - 21.24)^2}{21.24} \\
&= 4.71 + 4.82 + 4.38 + 4.48 = 18.39
\end{aligned}$$

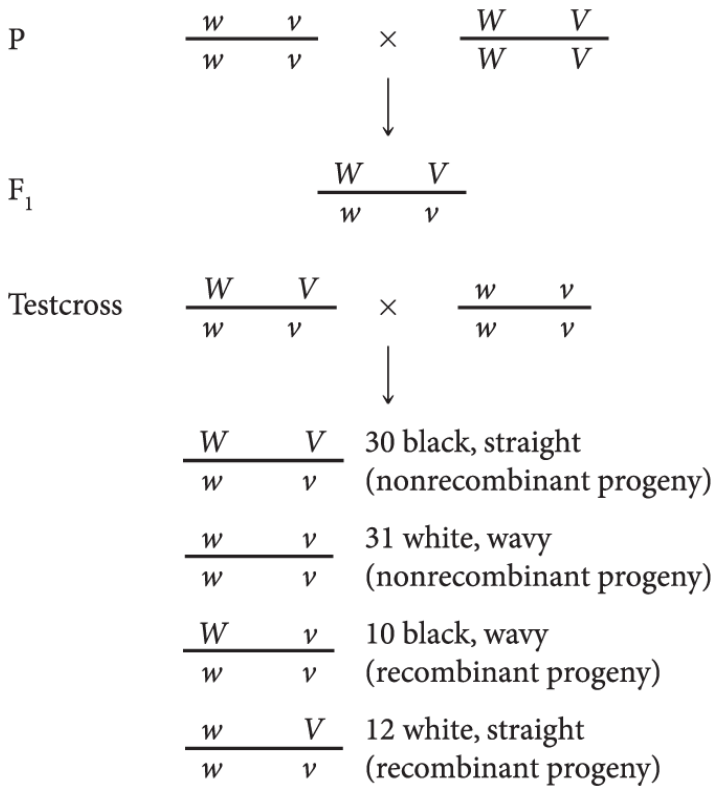
The degrees of freedom for the chi-square test of independence are df = (number of rows − 1) × (number of columns − 1) . There are two rows and two columns, so the degrees of freedom are

$$\text{df} = (2 - 1) \times (2 - 1) = 1 \times 1 = 1$$

In Table 3.5, the probability associated with a chi-square value of 18.39 and 1 degree of freedom is less than 0.005, indicating that chance is very unlikely to be responsible for the differences between the observed numbers and the numbers expected with independent assortment. The genes for coat color and hair type have therefore not assorted independently.

> **Recall:** The probability associated with the chi-square value is the probability that the difference between observed and expected values is due to chance.

b. To determine the recombination frequencies, identify the recombinant progeny. Using the notation for linked genes, write the crosses:



Description

The P generation has a guinea pig with small W and small V on both the chromosomes and another has big W and big V on both the chromosomes. The F subscript 1 progeny below has big W big V on the top chromosome and small W small V on the bottom chromosome. The testcross is between the F subscript 1 progeny and a guinea pig with small W small V on both the chromosomes. The resultant progeny obtained below is as follows: Progeny 1 has big W big V on the top chromosome and small W small V on the bottom chromosome. These are 30 and are black straight. The second progeny listed is small W small V on both the chromosomes.

These are 31 and are white wavy. The third progeny has big W small V on the top chromosome and small W small V on the bottom chromosome. These are 10 and are black wavy. The fourth progeny has small big V on the top chromosome and small W small V on the bottom chromosome. These are 12 and are white straight. The first 2 are nonrecombinant progeny, and the last 2 are recombinant progeny.

$$\text{The recombination frequency} = \frac{10+12}{30+31+10+12} \times 100\% = \frac{22}{83} \times 100\% = 26.5$$

Recall: $\text{The recombination frequency} = \frac{\text{number of recombinant progeny}}{\text{total number of progeny}} \times 100\%.$

Problem 2

A series of two-point crosses were carried out among seven loci (*a*, *b*, *c*, *d*, *e*, *f*, and *g*), producing the following recombination frequencies. Using these recombination frequencies, map the seven loci, showing their linkage groups, the order of the loci in each linkage group, and the distances between the loci of each group:

Loci	Recombination frequency (%)	Loci	Recombination frequency (%)
a and *b*	10	*c* and *d*	50
a and *c*	50	*c* and *e*	8
a and *d*	14	*c* and *f*	50
a and *e*	50	*c* and *g*	12
a and *f*	50	*d* and *e*	50
a and *g*	50	*d* and *f*	50

b and *c*	50	*d* and *g*	50
b and *d*	4	*e* and *f*	50
b and *e*	50	*e* and *g*	18
b and *f*	50	*f* and *g*	50
b and *g*	50		

» Solution Strategy

What information is required in your answer to the problem?

The linkage groups for the seven loci, the order of the loci within each linkage group, and the map distances between the loci.

What information is provided to solve the problem?

Recombination frequencies for each pair of loci.

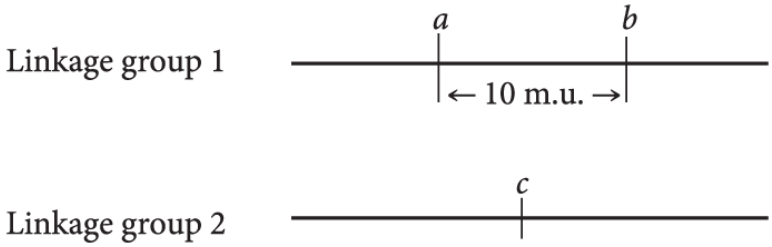
For help with this problem, review:

Constructing a Genetic Map with a Two-Point Testcross in Section 7.2.

» Solution Steps

To work this problem, remember that 1% recombination equals 1 map unit. The recombination frequency between *a* and *b* is 10%, so these two loci are in the same linkage group, approximately 10 m.u. apart.

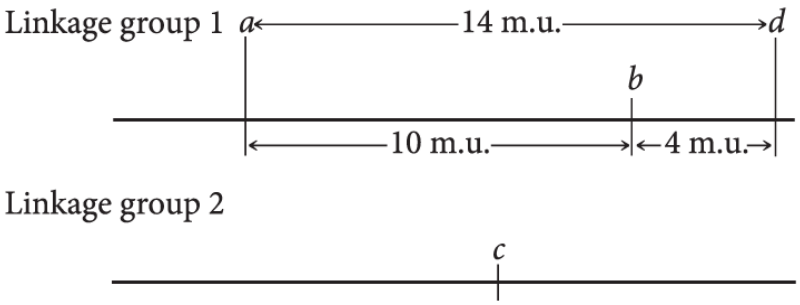
The recombination frequency between *a* and *c* is 50%, so *c* must lie in a second linkage group.



Description
The linkage group 1 shows distance between genes small A and small B is 10 m u. The linkage group 2 shows gene small C in the center.

Hint: A recombination frequency of 50% means that genes at the two loci are assorting independently (located in different linkage groups).

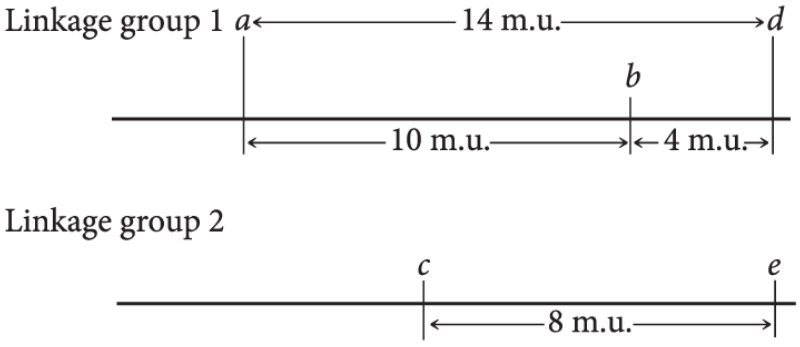
The recombination frequency between *a* and *d* is 14%, so *d* is located in linkage group 1. Is locus *d* 14 m.u. to the right or to the left of locus *a*? If *d* is 14 m.u. to the left of *a*, then the *b*-to-*d* distance should be 10 m.u. + 14 m.u. = 24 m.u. On the other hand, if *d* is to the right of *a*, then the distance between *b* and *d* should be 14 m.u. − 10 m.u. = 4 m.u. The *b*–*d* recombination frequency is 4%, so *d* is 14 m.u. to the right of *a*. The updated map is



Description
The first line is labeled linkage group 1 and shows genes small A and small B 10 m u away from each other. Gene small D is 4 m u to the right of gene small B. The distance between gene small A and small D is 14 m u. The second line is labeled linkage group 2 and shows gene small C in the center.

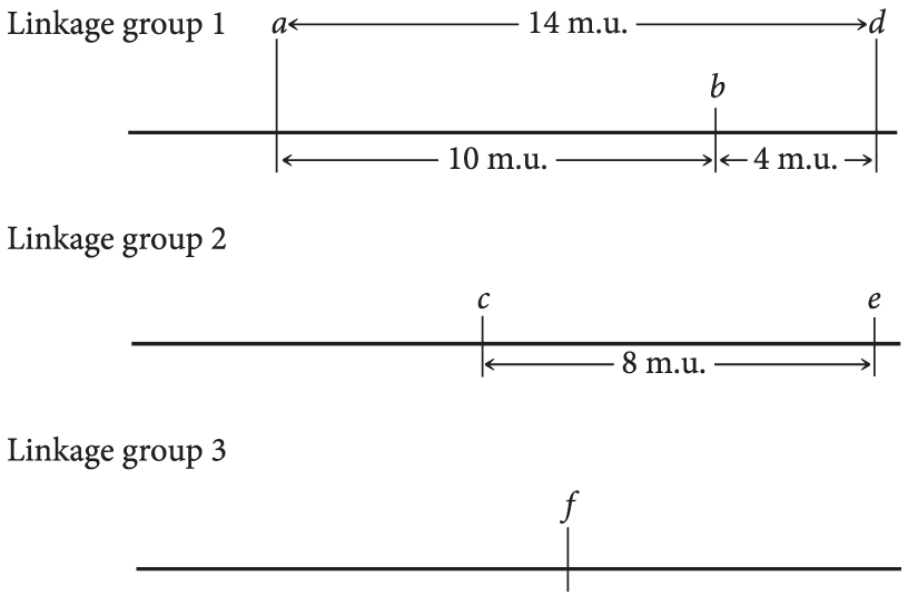
Hint: To determine whether locus *d* is to the right or the left of locus *a*, look at the *b*-to-*d* distance.

The recombination frequencies between each of loci *a*, *b*, and *d* and locus *e* are all 50%, so *e* is not in linkage group 1 with *a*, *b*, and *d*. The recombination frequency between *e* and *c* is 8%, so *e* is in linkage group 2:



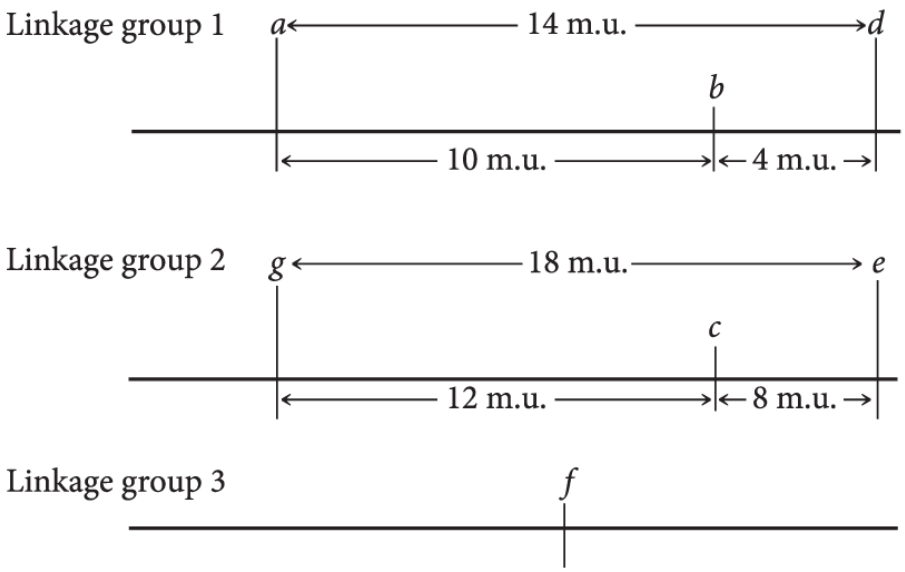
Description
The first line is labeled linkage group 1 and shows genes small A and small B 10 m u away from each other. Gene small D is an additional 4 m u to the right of gene small B. The distance between gene small A and small D is 14 m u. The second line is labeled linkage group 2 and shows gene small C in the middle and 8 m u to the right is gene small E.

There is 50% recombination between *f* and all the other genes, so *f* must belong to a third linkage group:



Description
The first line is labeled linkage group 1 and shows genes small A and small B 10 m u away from each other. Gene small D is 4 m u to the right of small B. The distance between gene small A and small D is 14 m u. The second line is labeled linkage group 2 and shows that gene small C in the middle and 8 m u to the right is gene small E. The third line is labeled linkage group 3 and has gene small F in the middle.

Finally, position locus *g* with respect to the other genes. The recombination frequencies between *g* and loci *a*, *b*, and *d* are all 50%, so *g* is not in linkage group 1. The recombination frequency between *g* and *c* is 12%, so *g* is a part of linkage group 2. To determine whether *g* is 12 m.u. to the right or left of *c*, consult the *g*–*e* recombination frequency. Because this recombination frequency is 18%, *g* must lie to the left of *c*:



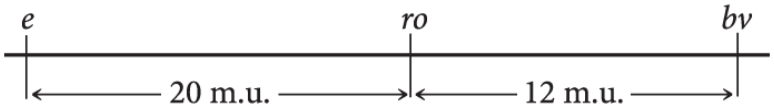
Description
The first line is labeled linkage group 1 and shows genes small A and small B 10 m u away from each other. Gene small D is 4 m u to the right of small B. The distance between gene small A and small D is 14 m u. The second line is labeled linkage group 2 and shows that gene small G is in extreme left, 12 m u to the right is gene small C and 8 m u to the right is gene small E. The distance between gene small G and small E is 18 m u. The third line is labeled linkage group 3 and has gene small F in the middle.

Note that the *g-to-e* distance (18 m.u.) is shorter than the sum of the *g-to-c* (12 m.u.) and *c-to-e* distances (8 m.u.) because of undetectable double crossovers between *g* and *e*.

Recall: Because some double crossovers may go undetected, the distance between two distant genes (such as *g* and *e*) may be less than the sum of shorter distances (such as *g* to *c* and *c* to *e*).

Problem 3

Ebony body color (*e*), rough eyes (*ro*), and brevis bristles (*bv*) are three recessive mutations that occur in fruit flies. The loci for these mutations have been mapped and are separated by the following map distances:



Description
The genes are from left to right are follows: gene small E is on extreme left, 20 m u to the right is gene small R small O, and 12 m u to the right is gene small B small V.

The interference between these genes is 0.4.

A fly with ebony body, rough eyes, and brevis bristles is crossed with a fly that is homozygous for the wild-type traits. The resulting F_1 females are test-crossed with males that have ebony body, rough eyes, and brevis bristles; 1800 progeny are produced. Give the expected numbers of phenotypes in the progeny of the testcross.

» Solution Strategy

What information is required in your answer to the problem?

The expected numbers of different progeny phenotypes produced by the testcross.

What information is provided to solve the problem?

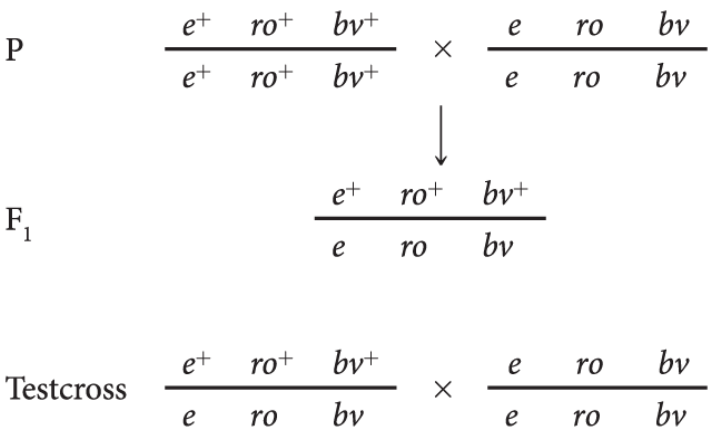
- The map distances among the three loci.
- The interference among the loci.
- A testcross is carried out and 1800 progeny are produced.

For help with this problem, review:

Constructing a Genetic Map with a Three-Point Testcross and the Worked Problem in Section 7.3.

» Solution Steps

The crosses are



Description
The P generation shows an individual with small E superscript positive small R small O superscript positive small B small V superscript positive on both the chromosomes crossed with an individual with small E small R small O small B small V on both the chromosomes. The F subscript 1 generation obtained below has small E superscript positive small R small O superscript positive small B small V superscript positive on one chromosome and small E small R small O small B small V on the other chromosome. A test cross below shows the F subscript 1 progeny crossed with an individual with small E small R small O small B small V on both the chromosomes.

In this case, we know that *ro* is the middle locus because the genes have been mapped. Eight classes of progeny will be produced by this cross:

e^+		ro^+	bv^+	nonrecombinant
e		*ro*	*bv*	nonrecombinant
e^+	/	*ro*	*bv*	single crossover between *e* and *ro*
e	/	ro^+	bv^+	single crossover between *e* and *ro*

e^+		ro^+	/	bv	single crossover between *ro* and *bv*
e		ro	/	bv^+	single crossover between *ro* and *bv*
e^+	/	ro	/	bv^+	double crossover
E	/	ro^+	/	bv	double crossover

Hint: The order of the genes is provided by the genetic map.

To determine the numbers of each type, use the map distances, starting with the double crossovers. The expected number of double crossovers is equal to the product of the single-crossover probabilities:

$$\begin{aligned} \text{expected number of double crossovers} &= 0.20 \times 0.12 \times 1800 \\ &= 43.2 \end{aligned}$$

However, there is some interference, so the observed number of double crossovers will be less than the expected. The interference is 1 – coefficient of coincidence, so the coefficient of coincidence is

$$\text{coefficient of coincidence} = 1 - \text{interference}$$

Recall: The presence of interference means that not all expected double crossovers will be observed.

The interference is given as 0.4, so the coefficient of coincidence equals 1 − 0.4 = 0.6. Recall that the coefficient of coincidence is

$$\text{coefficient of coincidence} = \frac{\text{number of observed double crossovers}}{\text{number of expected double crossovers}}$$

Rearranging this equation, we obtain

$$\begin{aligned}\text{number of observed double crossovers} &= \text{coefficient of coincidence} \times \text{number of expected double crossovers} \\ &= 0.6 \times 43.2 = 26\end{aligned}$$

A total of 26 double crossovers should be observed. Because there are two classes of double crossovers ($\underline{e^+ \ / \ ro \ / \ bv^+}$ and $\underline{e \ / \ ro^+ \ / \ bv}$,) we expect to observe 13 of each class.

Next, we determine the number of single-crossover progeny. The genetic map indicates that the distance between *e* and *ro* is 20 m.u., so 360 progeny (20% of 1800) are expected to have resulted from recombination between these two loci. Some of them will be single-crossover progeny, and some will be double-crossover progeny. We have already determined that the number of double-crossover progeny is 26, so the number of progeny resulting from a single crossover between *e* and *ro* is 360 − 26 = 334, which will be divided equally between the two single-crossover phenotypes ($\underline{e \ / \ ro^+ \ \ bv^+}$ and $\underline{e^+ \ / \ ro \ bv}$).

Hint: To obtain the number of single-crossover progeny, subtract the number of double crossover progeny from the total number that resulted from recombination.

The distance between *ro* and *bv* is 12 m.u., so the number of progeny resulting from recombination between these two genes is 0.12 × 1800 = 216. Again, some of these recombinants will be single-crossover progeny, and some will be double-crossover progeny. To determine the number of progeny resulting from a single crossover, subtract the double crossovers: 216 − 26 = 190. These single-crossover progeny will be divided between the two single-crossover phenotypes ($\underline{e^+ \ ro^+ \ / \ bv}$ and $\underline{e \ ro \ / \ bv^+}$), so there will be $^{190}/_{2} = 95$ of each of these phenotypes. The remaining progeny will be

nonrecombinants, and they can be obtained by subtraction: 1800 − 26 − 334 − 190 = 1250; there are two nonrecombinants ($e^+ \quad ro^+ \quad bv^+$ and $e \quad ro \quad bv$), so there will be ${}^{1250}/_{2}/625$ of each. The numbers of the various phenotypes are listed here:

e^+		ro^+		bv^+	625	nonrecombinant
e		ro		bv	625	nonrecombinant
e^+	/	ro		bv	167	single crossover between *e* and *ro*
e	/	ro^+		bv^+	167	single crossover between *e* and *ro*
e^+		ro^+	/	bv	95	single crossover between *ro* and *bv*
e		ro	/	bv^+	95	single crossover between *ro* and *bv*
e^+	/	ro	/	bv^+	13	double crossover
e	/	ro^+	/	bv	13	double crossover
Total					1800	

Hint: Don't forget to subtract the double crossovers from the total number of recombinants.

Hint: The number of nonrecombinants can be obtained by subtraction.

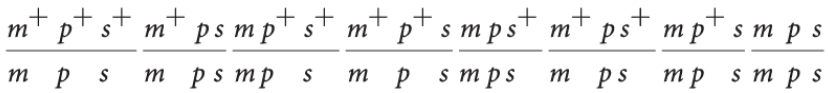
IMPORTANT TERMS

linked genes
linkage group
nonrecombinant (parental) gamete
nonrecombinant (parental) progeny
recombinant gamete
recombinant progeny

recombination frequency
coupling (cis) configuration
repulsion (trans) configuration
genetic map
physical map
map unit (m.u.)
centiMorgan (cM)
two-point testcross
three-point testcross
interference
coefficient of coincidence
mapping function
lod (logarithm of odds) score
genetic marker
linkage analysis
genome-wide association study
haplotype
linkage disequilibrium
single-nucleotide polymorphism (SNP)
somatic-cell hybridization
cell line
heterokaryon
deletion mapping

ANSWERS TO CONCEPT CHECKS

1. c
2. Repulsion
3. Genetic maps are based on rates of recombination; physical maps are based on physical distances.

4. 

Description
Each chromosome is represented by a straight, horizontal line. For first chromosome: the alleles above the line are small M superscript positive small P superscript positive small S superscript positive and alleles below the line are small M small P small S;

For second chromosome: the alleles above the line are small M superscript positive small P small S and alleles below the line are small M small P small S;

For third chromosome: the alleles above the line are small M small P superscript positive small S superscript positive and alleles below the line are small M small P small S;

For fourth chromosome: the alleles above the line are small M superscript positive small P superscript positive small S and alleles below the line are small M small P small S;

For fifth chromosome: the alleles above the line are small M small P small S superscript positive and alleles below the line are small M small P small S;

For sixth chromosome: the alleles above the line are small M superscript positive small P small S superscript positive and alleles below the line are small M small P small S;

For seventh chromosome: the alleles above the line are small M small P superscript positive small S and alleles below the line are small M small P small S;

For eighth chromosome: the alleles above and below the line are small M small P small S.

5. The *c* locus
6. b

Chapter 7 Assessment

COMPREHENSION QUESTIONS

Section 7.1

1. What does the term *recombination* mean? What are two causes of recombination?

Section 7.2

2. In a testcross for two genes, what types of gametes are produced with (a) complete linkage, (b) independent assortment, and (c) incomplete linkage?

3. What effect does crossing over have on linkage?

4. Why is the frequency of recombinant gametes always half the frequency of crossing over?

5. What is the difference between genes in coupling configuration and genes in repulsion? How does the arrangement of linked genes (whether they are in coupling or repulsion) affect the results of a genetic cross?

6. CONNECT Explain how Creighton and McClintock's experiments involving a strain of corn with an abnormal chromosome 9

demonstrated the chromosome theory of inheritance (see Chapter 3).

7. How would you test to see if two genes are linked?
8. What is the relationship between recombination frequency and a centiMorgan?
9. Why do calculated recombination frequencies between pairs of loci that are located far apart underestimate the true genetic distances between loci?

Section 7.3

10. Explain how to determine, using the numbers of progeny from a three-point cross, which of three linked loci is the middle locus.
11. What does the interference tell us about the effect of one crossover on another?
12. What is a lod score, and how is it calculated?

Section 7.4

13. List some of the methods for physically mapping genes, and explain how they are used to position genes on chromosomes.

APPLICATION QUESTIONS AND PROBLEMS

Section 7.2

14. CONNECT In the snail *Cepaea nemoralis*, an autosomal allele causing a banded shell (B^B) is recessive to the allele for an unbanded shell (B^O). Genes at a different locus determine the background color of the shell; here, yellow (C^Y) is recessive to brown (C^{Bw}). A banded, yellow snail is crossed with a homozygous brown, unbanded snail. The F_1 are then crossed with banded, yellow snails (a testcross).

a.

Picture Press/Getty Images.

What will the results of the testcross be if the loci that control banding and color are linked with no crossing over?

b. What will the results of the testcross be if the loci assort independently (see Chapter 3)?

c. What will the results of the testcross be if the loci are linked and 20 m.u. apart?

15. *In silkmoths (*Bombyx mori*), red eyes (*re*) and white-banded wings (*wb*) are encoded by two mutant alleles that are recessive to those that produce wild-type traits (re^+ and wb^+); these two genes are on the same chromosome. A moth homozygous for red eyes and white-banded wings is crossed with a moth homozygous for the wild-type traits. The F_1 have wild-type eyes and wild-type wings. The F_1 are crossed with moths that have red eyes and white-banded wings in a testcross. The progeny of this testcross are

wild-type eyes, wild-type wings	418
red eyes, wild-type wings	19
wild-type eyes, white-banded wings	16
red eyes, white-banded wings	426

a. What phenotypic proportions would be expected if the genes for red eyes and for white-banded wings were located on different chromosomes?

b. What is the rate of recombination between the gene for red eyes and the gene for white-banded wings?

16. CONNECT *In 1916, Dorothy Osborn suggested that pattern baldness (the common type of baldness seen in most males) is a sex-influenced trait (see Chapter 5) that is dominant in males and recessive in females. Other research has found that pattern baldness is influenced by genes on the X chromosome. Would

you expect to see independent assortment between genetic markers on the X chromosome and pattern baldness if (a) pattern baldness is sex-influenced and (b) if pattern baldness is X-linked recessive? Explain your answer. (Research has also identified variation at autosomal genes that is associated with pattern baldness.)

17. *A geneticist discovers a new mutation in *D. melanogaster* that causes the flies to shake and quiver. She calls this mutation *quiver* (*qu*) and determines that it is due to an autosomal recessive gene. She wants to determine whether the gene encoding the quiver phenotype is linked to the recessive gene encoding vestigial (reduced) wings (*vg*). She crosses a fly homozygous for quiver and vestigial traits with a fly homozygous for the wild-type traits and then uses the resulting F_1 females in a testcross. She obtains the following flies from this testcross:

vg^+	qu^+	230
vg	*qu*	224
vg	qu^+	97
vg^+	*qu*	99
Total		650

Are the genes that cause vestigial wings and the quiver phenotype linked? Do a chi-square test of independence to determine whether the genes have assorted independently.

18. *In cucumbers, heart-shaped leaves (*hl*) are recessive to normal leaves (*Hl*), and having numerous fruit spines (*ns*) is recessive to

having few fruit spines (*Ns*). The genes for leaf shape and for number of spines are located on the same chromosome; findings from mapping experiments indicate that they are 32.6 m.u. apart. A cucumber plant having heart-shaped leaves and numerous spines is crossed with a plant that is homozygous for normal leaves and few spines. The F_1 are crossed with plants that have heart-shaped leaves and numerous spines. What phenotypes and phenotypic proportions are expected in the progeny of this cross?

19. In tomatoes, tall (*D*) is dominant over dwarf (*d*), and smooth fruit (*P*) is dominant over pubescent fruit (*p*), which is covered with fine hairs. A farmer has two tall and smooth-fruit tomato plants, which we will call plant A and plant B. The farmer crosses plants A and B with the same dwarf and pubescent-fruitx plant and obtains the following numbers of progeny:

	Progeny of	
	Plant A	**Plant B**
Dd Pp	122	2
Dd pp	6	82
dd Pp	4	82
dd pp	124	4

a. What are the genotypes of plant A and plant B?

b. Are the loci that determine the height of the plant and pubescence linked? If so, what is the rate of recombination between them?

c. Explain why different proportions of progeny are produced when plant A and plant B are crossed with the same dwarf and pubescent-fruit plant.

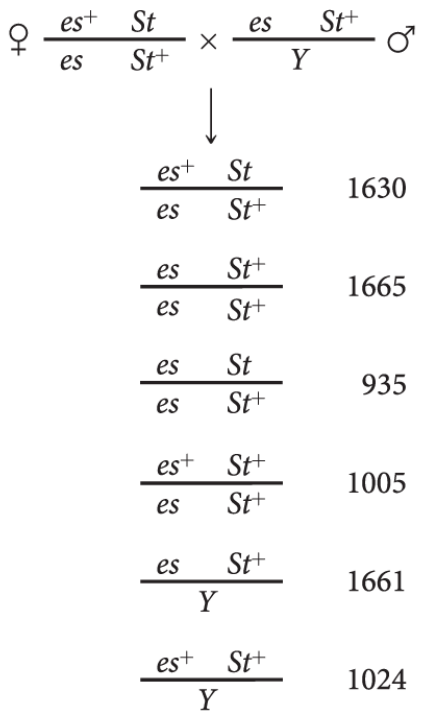
20. Alleles *A* and *a* reside at a locus on the same chromosome as a locus with alleles *B* and *b*. *Aa Bb* is crossed with *aa bb*, and the following progeny are produced:

Aa Bb	5
Aa bb	45
aa Bb	45
aa bb	5

What conclusion can be drawn about the arrangement of the genes on the chromosome in the *Aa Bb* parent?

21.

Daniel McDonald and Nancy Peer determined that eyespot (a clear spot in the center of the eye) in flour beetles is caused by an X-linked allele (*es*) that is recessive to the allele for the absence of eyespot (es^+). They conducted a series of crosses to determine the distance between the gene for eyespot and a dominant X-linked gene for striped (*St*), which causes white stripes on females and acts as a recessive lethal (is lethal when homozygous in females or hemizygous in males). The following cross was carried out (D. J. McDonald and N. J. Peer. 1961. *Journal of Heredity* 52:261–264).



Description

The female has small E small S superscript positive and big S small T on the top chromosome and small E small S and big S small T superscript positive on the bottom chromosome. The male has small E small S and big S small T superscript positive on the top chromosome and a Y on the bottom chromosome. The male and female are crossed and the numbers of progeny and their allele combinations obtained below are as follows: There are 1630 progenies with small E small S superscript positive and big S small T on the top chromosome and small E small S and big S small T superscript positive on the bottom chromosome. There are 1665 progenies with small E small S and big S small T superscript positive on the top chromosome and small E small S and big S small T superscript positive on the bottom chromosome. There are 935 progenies with small E small S and big S small T on the top chromosome and small E small S and big S small T superscript positive on the bottom chromosome. There are 1005 progenies with small E small S superscript positive and big S small T superscript positive on the top chromosome and small E small S and big S small T superscript positive on the bottom chromosome. There are 1661 progenies with small E small S and big S small T superscript positive on the top chromosome and big Y on the bottom chromosome. There are 1024 progenies with small E small S superscript positive and big S small T superscript positive on the top chromosome and big Y on the bottom chromosome.

a. Which progeny are the recombinants, and which progeny are the nonrecombinants?

b. Calculate the recombination frequency between *es* and *St*.

c. Are some potential genotypes missing among the progeny of the cross? If so, which ones, and why?

22. *Recombination frequencies between three loci in corn are shown in the following table:

Loci	Recombination frequency (%)
R and W_2	17
R and L_2	35
W_2 and L_2	18

What is the order of the genes on the chromosome?

23. In tomatoes, dwarf (*d*) is recessive to tall (*D*), and opaque (light-green) leaves (*op*) are recessive to green leaves (*Op*). The loci that determine height and leaf color are linked and separated by a distance of 7 m.u. For each of the following crosses, determine the phenotypes and proportions of progeny produced.

a. $\frac{D \quad Op}{d \quad op} \times \frac{d \quad op}{d \quad Op}$

b. $\frac{D \quad op}{d \quad Op} \times \frac{d \quad op}{d \quad op}$

c. $\frac{D \quad Op}{d \quad op} \times \frac{D \quad Op}{d \quad op}$

d. $\frac{D \quad op}{d \quad Op} \times \frac{D \quad op}{d \quad Op}$

24. In German cockroaches, bulging eyes (*bu*) are recessive to normal eyes (bu^+), and curved wings (*cv*) are recessive to straight wings (cv^+). These two traits are encoded by autosomal genes that are linked. A cockroach has genotype $bu^+bu\ cv^+cv$, and the genes are in repulsion. Which of the following sets of genes will be found in the most common gametes produced by this cockroach?

    a. $bu^+\ cv^+$

    b. *bu cv*

    c. bu^+bu

    d. cv^+cv

    e. $bu\ cv^+$

    Explain your answer.

25. In *D. melanogaster*, ebony body (*e*) and rough eyes (*ro*) are encoded by autosomal recessive genes found on chromosome 3; they are separated by 20 m.u. The gene that encodes forked bristles (*f*) is X-linked recessive and assorts independently of *e* and *ro*. Give the phenotypes of progeny and their expected proportions when a female of each of the following genotypes is test-crossed with a male.

    a. $\frac{e^+ \quad ro^+}{e \quad ro} \quad \frac{f^+}{f}$

b. $\frac{e^+ \quad ro}{e \quad ro^+} \quad \frac{f^+}{f}$

26. DATA ANALYSIS Honeybees have haplodiploid sex determination: queens (females) are diploid, developing from fertilized eggs, whereas drones (males) are haploid, developing from unfertilized eggs. Otto Mackensen studied linkage relations among eight mutations in honeybees (O. Mackensen. 1958. *Journal of Heredity* 49:99–102). The following table gives the results of two of Mackensen's crosses, including three recessive mutations: *cd* (cordovan body color), *h* (hairless), and *ch* (chartreuse eye color).

Queen genotype	Phenotypes of drone (male) progeny
$\frac{cd \quad h^+}{cd^+ \quad h}$	294 cordovan, 236 hairless, 262 cordovan and hairless, 289 wild type
$\frac{h \quad ch^+}{h^+ \quad ch}$	3131 hairless, 3064 chartreuse, 96 chartreuse and hairless, 132 wild type

a. Only the genotype of the queen is given. Why is the genotype of the male parent not needed for mapping these genes? Would the genotype of the male parent be required if we examined female progeny instead of male progeny?

b. Determine the nonrecombinant and recombinant progeny for each cross, and calculate the map distances between *cd*, *h*, and *ch*. Draw a linkage map illustrating the linkage arrangements among these three genes.

27. Perform a chi-square test of independence on the data provided in Figure 7.2 to determine if the genes for flower color and pollen shape in sweet peas are assorting independently. Give the chi-

square value, degrees of freedom, and associated probability. What conclusion would you draw about the independent assortment of these genes?

28. *A series of two-point crosses were carried out among seven loci (*a, b, c, d, e, f,* and *g*), producing the following recombination frequencies. Map the seven loci, showing their linkage groups, the order of the loci in each linkage group, and the distances between the loci of each group.

Loci	Recombination frequency (%)	Loci	Recombination frequency (%)
a and *b*	50	*c* and *d*	50
a and *c*	50	*c* and *e*	26
a and *d*	12	*c* and *f*	50
a and *e*	50	*c* and *g*	50
a and *f*	50	*d* and *e*	50
a and *g*	4	*d* and *f*	50
b and *c*	10	*d* and *g*	8
b and *d*	50	*e* and *f*	50
b and *e*	18	*e* and *g*	50
b and *f*	50	*f* and *g*	50
b and *g*	50		

29.

R. W. Allard and W. M. Clement determined recombination rates for a series of genes in lima beans (R. W. Allard and W. M. Clement. 1959. *Journal of Heredity* 50:63–67). The following table lists paired recombination frequencies for eight of the loci (*D, Wl, R, S,* L_1, *Ms, C,* and *G*) that they mapped. On the basis of these data, draw a series of genetic maps for the different linkage groups of the genes, indicating the distances between the genes. Keep in

mind that these frequencies are estimates of the true recombination frequencies and that some error is associated with each estimate. An asterisk beside a recombination frequency indicates that the recombination frequency is significantly different from 50%.

Recombination frequencies (%) among seven loci in lima beans

	Wl	*R*	*S*	L_1	*Ms*	*C*	*G*
D	2.1*	39.3*	52.4	48.1	53.1	51.4	49.8
Wl		38.0*	47.3	47.7	48.8	50.3	50.4
R			51.9	52.7	54.6	49.3	52.6
S				26.9*	54.9	52.0	48.0
L_1					48.2	45.3	50.4
Ms						14.7*	43.1
C							52.0

* *Significantly different from 50%.*

30. CONNECT A genetics student conducts some genetic crosses with *Drosophila* and finds that two phenotypes—purple eyes and dark body—tend to be inherited together. How could the student determine whether this association between dark eyes and dark body color is due to pleiotropy (see Chapter 5) or linkage?

Section 7.3

31. DATA ANALYSIS *Raymond Popp studied linkage among genes for pink eye (*p*), shaker-1 (*sh*-1, which causes circling behavior, head tossing, and

deafness), and hemoglobin (*Hb*) in mice (R. A. Popp. 1962. *Journal of Heredity* 53:73–80). He performed a series of testcrosses, in which mice heterozygous for pink eye, shaker-1, and hemoglobin 1 and 2 were crossed with mice that were homozygous for pink eye, shaker-1, and hemoglobin 2.

$$\frac{P\ Sh-1\ Hb^{1}}{p\ sh-1\ Hb^{2}} \times \frac{p\ sh-1\ Hb^{2}}{p\ sh-1\ Hb^{2}}$$

The following progeny were produced:

Progeny genotype	Number
$\frac{p\ sh-1\ Hb^{2}}{p\ sh-1\ Hb^{2}}$	274
$\frac{P\ Sh-1\ Hb^{1}}{p\ sh-1\ Hb^{2}}$	320
$\frac{P\ sh-1\ Hb^{2}}{p\ sh-1\ Hb^{2}}$	57
$\frac{p\ Sh-1\ Hb^{1}}{p\ sh-1\ Hb^{2}}$	45
$\frac{P\ Sh-1\ Hb^{1}}{p\ sh-1\ Hb^{2}}$	6
$\frac{p\ sh-1\ Hb^{2}}{p\ sh-1\ Hb^{2}}$	5

$\frac{p\ Sh - 1\ Hb^2}{p\ sh - 1\ Hb^2}$	0
$\frac{P\ sh - 1\ Hb^1}{p\ sh - 1\ Hb^2}$	1
Total	708

a. Determine the order of these genes on the chromosome.

b. Calculate the map distances between the genes.

c. Determine the coefficient of coincidence and the interference among these genes.

32. *Waxy endosperm (*wx*), shrunken endosperm (*sh*), and yellow seedlings (*v*) are encoded by three recessive genes in corn that are linked on chromosome 5. A corn plant homozygous for all three recessive alleles is crossed with a plant homozygous for all the dominant alleles. The resulting F_1 are then crossed with a plant homozygous for the recessive alleles in a three-point testcross. The progeny of the testcross are

wx	*sh*	*V*	87
Wx	*Sh*	*v*	94
Wx	*Sh*	*V*	3,479
wx	*sh*	*v*	3,478
Wx	*sh*	*V*	1,515
wx	*Sh*	*v*	1,531
wx	*Sh*	*V*	292
Wx	*sh*	*v*	280

Total	10,756

a. Determine the order of these genes on the chromosome.

b. Calculate the map distances between the genes.

c. Determine the coefficient of coincidence and the interference among these genes.

33.

Priscilla Lane and Margaret Green studied the linkage relations of three genes affecting coat color in mice: mahogany (*mg*), agouti (*a*), and ragged (*Rg*). They carried out a series of three-point crosses, mating mice that were heterozygous at all three loci with mice that were homozygous for the recessive alleles at these loci (P. W. Lane and M. C. Green. 1960. *Journal of Heredity* 51:228–230). The following table lists the progeny of the testcrosses:

a	*Rg*	+	1
+	+	*mg*	1
a	+	+	15
+	*Rg*	*mg*	9
+	+	+	16
a	*Rg*	*mg*	36
a	+	*mg*	76
+	*Rg*	+	69
Total			223

Note: + represents a wild-type allele.

a. Determine the order of the loci that encode mahogany, agouti, and ragged on the chromosome; the map distances between

them; and the coefficient of coincidence and the interference among these genes.

b. Draw a picture of the two chromosomes in the triply heterozygous mice used in the testcrosses, indicating which of the alleles are present on each chromosome.

34. Fine spines (*s*), smooth fruit (*tu*), and uniform fruit color (*u*) are three recessive traits in cucumbers, the genes for which are linked on the same chromosome. A cucumber plant heterozygous for all three traits is used in a testcross, and the following progeny are produced by this testcross:

S	*U*	*Tu*	2
s	*u*	*Tu*	70
S	*u*	*Tu*	21
s	*u*	*tu*	4
S	*U*	*tu*	82
s	*U*	*tu*	21
s	*U*	*Tu*	13
S	*u*	*tu*	17
Total			230

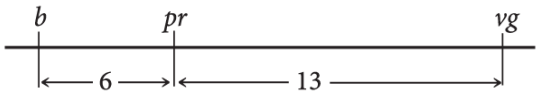
a. Determine the order of these genes on the chromosome.

b. Calculate the map distances between the genes.

c. Determine the coefficient of coincidence and the interference among these genes.

d. List the genes found on each chromosome in the parents used in the testcross.

35. In *D. melanogaster*, black body (*b*) is recessive to gray body (b^+), purple eyes (*pr*) are recessive to red eyes (pr^+), and vestigial wings (*vg*) are recessive to normal wings (vg^+). The loci encoding these traits are linked, with the following map distances:



Description
The gene map shows gene small B on the extreme left side. Gene small P small R is 6 m u to the right of gene small B, and gene small V small G is 13 m u to the right of gene small P small R.

The interference among these genes is 0.5. A fly with a black body, purple eyes, and vestigial wings is crossed with a fly homozygous for a gray body, red eyes, and normal wings. The female progeny are then crossed with males that have a black body, purple eyes, and vestigial wings. If 1000 progeny are produced by this testcross, what will be the phenotypes and proportions of the progeny?

36. *Sepia eyes*, *spineless bristles*, and *striped body* are three recessive mutations in *Drosophila* found on chromosome 3. A genetics student crosses a fly homozygous for the alleles encoding sepia eyes, spineless bristles, and striped body with a fly homozygous for the wild-type alleles—encoding red eyes, normal bristles, and solid body. The female progeny are then test-crossed with males that have sepia eyes, spineless bristles, and striped body. Assume that the interference between these genes is 0.2 and that 400 progeny

flies are produced by the testcross. Based on the map distances provided in Figure 7.15, predict the phenotypes and proportions of the progeny resulting from the testcross.

37. Shown below are eight DNA sequences from different individuals.

	Nucleotide position														
	1				5				10				15		
Sequence 1	T	C	T	G	G	A	T	C	A	T	C	A	C	A	T . . .
Sequence 2	A	C	A	G	C	A	T	C	A	T	T	A	C	G	T . . .
Sequence 3	T	C	A	G	G	A	T	C	A	T	T	A	C	T	A . . .
Sequence 4	T	C	A	G	G	A	T	C	A	T	T	A	C	A	T . . .
Sequence 5	A	C	A	G	C	A	T	C	A	T	T	A	C	G	T . . .
Sequence 6	T	C	T	G	G	A	T	C	A	T	C	A	C	A	T . . .
Sequence 7	T	C	A	G	G	A	T	C	A	T	T	A	C	A	T . . .
Sequence 8	A	C	A	G	C	A	T	C	A	T	T	A	C	G	T . . .

Description
The illustration shows the following information from top to bottom:

Sequence 1: T (Position 1), C, T, G, G (Position 5), A, T, C, A, T (Position 10), C, A, C, A, T (Position 15).

Sequence 2: A (Position 1), C, A, G, C (Position 5), A, T, C, A, T (Position 10), T, A, C, G, T (Position 15).

Sequence 3: T (Position 1), C, A, G, G (Position 5), A, T, C, A, T (Position 10), T, A, C, T, A (Position 15).

Sequence 4: T (Position 1), C, A, G, G (Position 5), A, T, C, A, T (Position 10), T, A, C, A, T (Position 15).

Sequence 5: A (Position 1), C, A, G, C (Position 5), A, T, C, A, T (Position 10), T, A, C, G, T (Position 15).

Sequence 6: T (Position 1), C, T, G, G (Position 5), A, T, C, A, T (Position 10), C, A, C, A, T (Position 15).

Sequence 7: T (Position 1), C, A, G, G (Position 5), A, T, C, A, T (Position 10), T, A, C, A, T (Position 15).

Sequence 8: A (Position 1), C, A, G, C (Position 5), A, T, C, A, T (Position 10), T, A, C, G, T (Position 15).

a. Give the nucleotide positions of all single-nucleotide polymorphisms (SNPs; nucleotide positions where individuals vary in a single nucleotide base) in these sequences.

b. How many different haplotypes (sets of linked variants) are found in these eight sequences?

c. Give the haplotype of each sequence by listing the specific bases at each variable position in that particular haplotype. (Hint: See **Figure 20.8**.)

38. *A group of geneticists are interested in identifying genes that may play a role in susceptibility to asthma. They study the inheritance of genetic markers in a series of families that have two or more asthmatic children. They find an association between the presence or absence of asthma and a genetic marker on the short arm of chromosome 20 and calculate a lod score of 2 for this association. What does this lod score indicate about genes that may influence asthma?

Section 7.4

39. A panel of cell lines was created by human–mouse somatic-cell hybridization. Each line was examined for the presence of human chromosomes and for the production of an enzyme. The following results were obtained:

		Human chromosomes											
Cell line	**Enzyme**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**17**	**22**
A	−	+	−	−	−	+	−	−	−	−	−	+	−
B	+	+	+	−	−	−	−	−	+	−	−	+	+
C	−	+	−	−	−	+	−	−	−	−	−	−	+
D	−	−	−	−	+	−	−	−	−	−	−	−	−
E	+	+	−	−	−	−	−	−	+	−	+	+	−

Description
The column headers are as follows: cell line, enzyme, the numbers 1 to 10, 17, and 22.

The row entries are as follows:

Row 1: A, negative, positive, negative, negative, negative, positive, negative, negative, negative, negative, negative, positive, and negative.

Row 2: B, positive, positive, positive, negative, negative, negative, negative, negative, positive, negative, negative, positive, and positive.

Row 3: C, negative, positive, negative, negative, negative, positive, negative, negative, negative, negative, negative, negative, and positive.

Row 4: D, negative, negative, negative, negative, positive, negative, negative, negative, negative, negative, negative, negative, and negative.

Row 5: E, positive, positive, negative, negative, negative, negative, negative, negative, positive, negative, negative, positive, and negative.

On the basis of these results, which chromosome has the gene that encodes the enzyme?

40. *A panel of cell lines was created by human–mouse somatic-cell hybridization. Each cell line was examined for the presence of human chromosomes and for the production of three enzymes. The following results were obtained:

Cell line	Enzyme			Human chromosomes							
	1	2	3	4	8	9	12	15	16	17	22	X
A	+	−	+	−	−	+	−	+	+	−	−	+
B	+	−	−	−	−	+	−	−	+	+	−	−
C	−	+	+	+	−	−	−	−	−	+	−	+
D	−	+	+	+	+	−	−	−	+	−	−	+

Description
The column headers are as follows: cell line, the numbers 1 to 9, 12, 15 to 17, 22, and X; where headers 1 to 4 are enzymes and the rest from 8 to X are human chromosomes.

Row entries are as follows:

Row 1: A, positive, negative, positive, negative, negative, positive, negative, positive, positive, negative, negative, and positive.

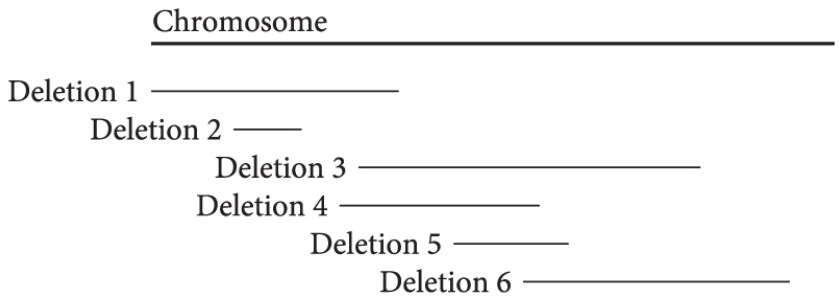
Row 2: B, positive, negative, negative, negative, negative, positive, negative, negative, positive, positive, negative, and negative.

Row 3: C, negative, positive, positive, positive, negative, negative, negative, negative, negative, positive, negative, and positive.

Row 4: D, negative, positive, positive, positive, positive, negative, negative, negative, positive, negative, negative, and positive.

On the basis of these results, give the chromosomal locations of the genes encoding enzyme 1, enzyme 2, and enzyme 3.

41. *The locations of six deletions have been mapped to a *Drosophila* chromosome, as shown in the following deletion map. Recessive mutations *a*, *b*, *c*, *d*, *e*, and *f* are known to be located in the same region as the deletions, but the order of the mutations on the chromosome is not known.



When flies homozygous for the recessive mutations are crossed with flies heterozygous for the deletions, the following results are obtained, in which "m" represents a mutant phenotype and a plus sign (+) represents the wild type. On the basis of these data, determine the relative order of the six mutant genes on the chromosome.

	Mutations					
Deletion	***a***	***b***	***c***	***d***	***e***	***f***
1	m	+	m	+	+	m
2	m	+	+	+	+	+
3	+	m	m	m	m	+
4	+	+	m	m	m	+
5	+	+	+	m	m	+
6	+	m	+	m	+	+

CHALLENGE QUESTIONS

Section 7.5

42. Transferrin is a blood protein that is encoded by the transferring locus (*Trf*). In house mice, the two alleles at this locus (Trf^a and Trf^b) are codominant and encode three types of transferrin:

Genotype	Phenotype
Trf^a/Trf^a	Trf-a
Trf^a/Trf^b	Trf-ab
Trf^b/Trf^b	Trf-b

The dilution locus, found on the same chromosome, determines whether the color of a mouse is diluted or full; an allele for dilution (d) is recessive to an allele for full color (d^+):

Genotype	Phenotype
d^+d^+	d^+ (full color)
d^+d	d^+ (full color)
Dd	d (dilution)

Donald Shreffler conducted a series of crosses to determine the map distance between the transferrin locus and the dilution locus (D. C. Shreffler. 1963. *Journal of Heredity* 54:127–129). The following table presents a series of crosses carried out by Shreffler and the progeny resulting from these crosses.

		Progeny phenotypes				
		d^+	d^+	d	d	
Cross	♂ ♀	**Trf-ab**	**Trf-b**	**Trf-ab**	**Trf-b**	**Total**

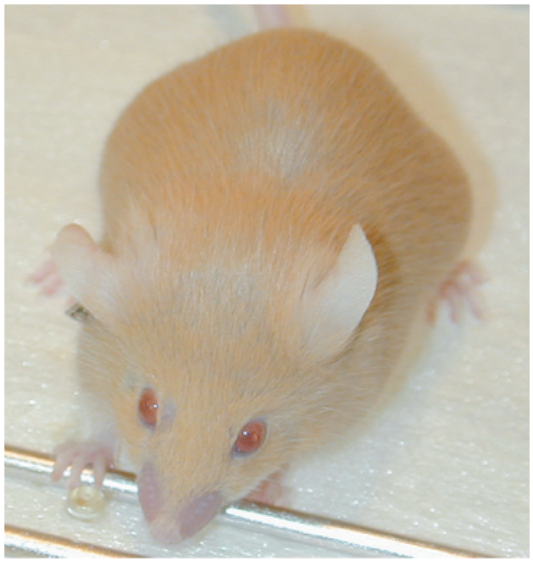
1	$\frac{d^{+}Trf^{a}}{d\ Trf^{b}} \times \frac{d\ Trf^{b}}{d\ Trf^{b}}$	32	3	6	21	62
2	$\frac{d\,Trf^{b}}{d\,Trf^{b}} \times \frac{d^{+}\,Trf^{a}}{d\ Trf^{b}}$	16	0	2	20	38
3	$\frac{d^{+}\,Trf^{a}}{d\,Trf^{b}} \times \frac{d\ Trf^{b}}{d\ Trf^{b}}$	35	9	4	30	78
4	$\frac{d\,Trf^{b}}{d\,Trf^{b}} \times \frac{d^{+}\ Trf^{a}}{d\ Trf^{b}}$	21	3	2	19	45
5	$\frac{d^{+}\,Trf^{b}}{d\,Trf^{a}} \times \frac{d\ Trf^{b}}{d\ Trf^{b}}$	8	29	22	5	64
6	$\frac{d\,Trf^{b}}{d\,Trf^{b}} \times \frac{d^{+}\,Trf^{b}}{d\ Trf^{a}}$	4	14	11	0	29

a. Calculate the recombination frequency between the *Trf* and the *d* loci by using the pooled data from all the crosses.

b. Which crosses represent recombination in male gamete formation, and which crosses represent recombination in female gamete formation?

c. On the basis of your answer to part *b*, calculate the frequency of recombination among male parents and female parents separately.

d. Are the rates of recombination in males and females the same? If not, what might produce the difference?



L. Montoliu, W. S. Oetting, D. C. Bennett. Color Genes. March 2010. European Society for Pigment Cell Research. (http:// www.espcr.org/ micemut) 03/2013

A mouse with the dilution trait.

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 7.1

1. Species A has $2n = 10$ chromosomes. Species B has $2n = 40$ chromosomes. On average, will two randomly selected genes from species A be more likely, less likely, or equally likely to assort independently than two randomly selected genes from species B? Explain your reasoning.

Section 7.2

2. The recombination frequency between genes *a* and *b* is 20%. What is the frequency of crossing over between genes *a* and *b*? Explain your reasoning.

3. Is it possible to have a recombination frequency of 80% between two genes? Is it possible for two genes to be separated by 80 map units? Why or why not?

4. The rate of crossing over between two linked genes (*r* and *w*) is 0.44. The following cross is carried out: *Rr Ww* × *rr ww*. What proportion of the gametes of the *Rr Ww* parent were recombinant gametes? Explain your reasoning, and state any assumptions you made.

Section 7.3

5. Why is interference important? Why do we calculate it in a three-point cross? Why don't we calculate interference in a two-point cross?

6. Three recessive mutations in *D. melanogaster—roughoid* (*ru*, small rough eyes), *javelin* (*jv*, cylindrical bristles), and *sepia eyes* (*se*, dark brown eyes)—are linked. A three-point cross was carried out and the following progeny obtained:

jv^+	ru^+	se^+	37
jv^+	ru^+	*se*	2
jv^+	*ru*	*se*	14

jv^+	*ru*	se^+	146
jv	*ru*	se^+	2
jv	*ru*	*se*	35
jv	ru^+	*se*	154
jv	ru^+	se^+	10

a. Determine the order of the genes on the chromosome.

b. Determine which progeny contain single crossovers and which contain double crossovers, and indicate where among the genes the crossovers occurred.

c. Calculate the map distances among the genes.

d. Calculate the coefficient of coincidence and interference among the genes.

7. Compare and contrast linkage analysis and genome-wide association studies. How are they similar? How are they different?

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Create a concept map that outlines the results obtained from the genome-wide association study of fear and aggression in dogs.

Section 7.1

2. What differences are expected in the outcomes of crosses involving linked genes and crosses involving genes that assort independently? Draw a concept map to illustrate these differences.

Section 7.2

3. Create a concept map that outlines the use of recombination to map genes. Include the following terms: crossing over, linked genes, unlinked genes, recombination, independent assortment, gene mapping, recombination frequency, and linkage group.

Section 7.3

4. Draw a concept map that outlines the procedure for mapping genes using a three-point cross.

5. Create a concept map that illustrates the concept of crossover interference. Include at least the following terms in your concept map: single crossover frequency, observed number of double crossovers, expected number of double crossovers, coefficient of coincidence, interference, positive interference, and negative interference.

Section 7.4

6. What are the different physical methods used for mapping genes, and what are their methodologies? Create a map that includes the three physical methods outlined in this chapter (somatic cell hybridization, deletion mapping, and in situ hybridization), the

information each method provides, and how each differs in the methodology employed in mapping.

Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 8

Chromosome Variation

S_Photo/Shutterstock.

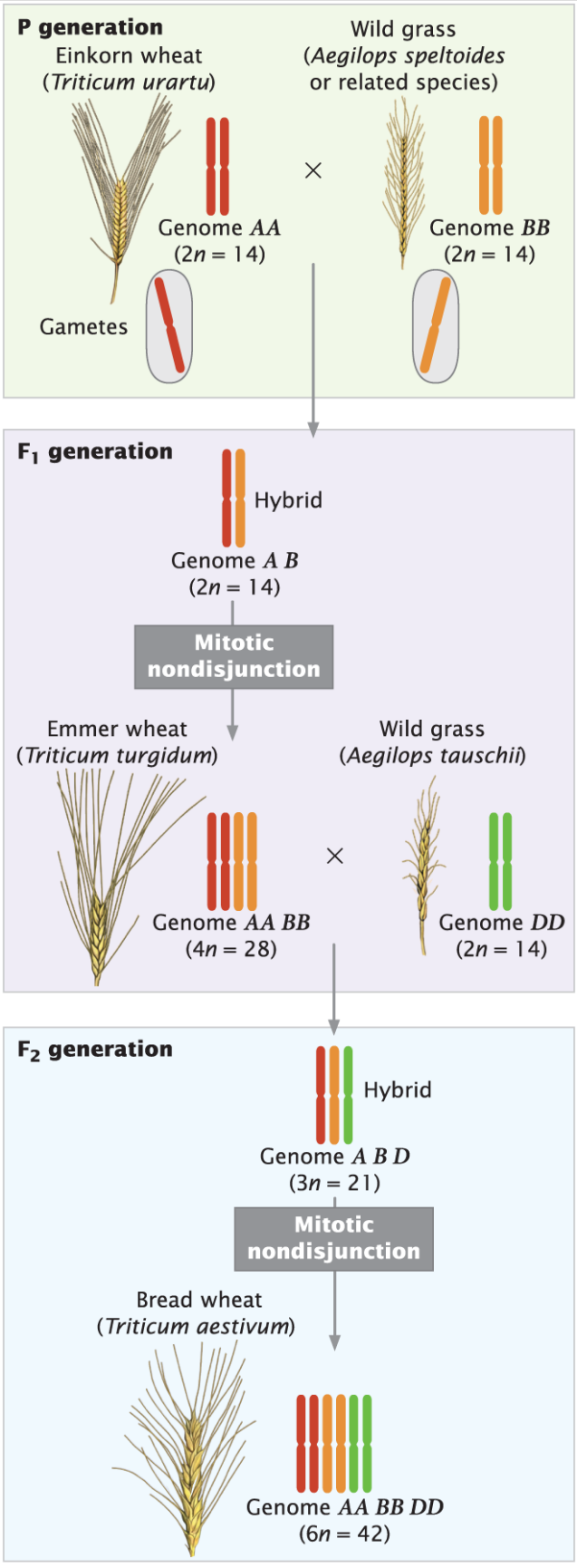
Wheat, the most widely grown food crop on Earth, has a genome consisting of chromosomes from three different species. The complete genome of wheat was sequenced in 2017.

The Complex Genome of Wheat

Nothing is more pleasing than the aroma and taste of freshly baked bread. Indeed, cooking, sharing, and eating bread has cultural and religious significance for billions of people around the world. The

primary constituent of bread—wheat—is the most widely grown crop on Earth, and foods made from wheat provide almost 20% of all calories consumed by humans worldwide.

Wheat has a highly complex genome that is a product of its unique evolutionary history. Most eukaryotic organisms are diploid ($2n$), consisting of two copies of genetic information, one inherited from each parent. Other organisms, such as fungi, are haploid (n), with a single copy of genetic information. But wheat is hexaploid ($6n$), with six copies of genetic information derived from three different species (**Figure 8.1**).


P generation
Einkorn wheat
(Triticum urartu)
Wild grass
(Aegilops speltoides
or related species)
Genome AA
(2n = 14)
Genome BB
(2n = 14)
Gametes
F1 generation
Hybrid
Genome A B
(2n = 14)
Mitotic
nondisjunction
Emmer wheat
(Triticum turgidum)
Wild grass
(Aegilops tauschii)
Genome AA BB
(4n = 28)
Genome DD
(2n = 14)
F2 generation
Hybrid
Genome A B D
(3n = 21)
Mitotic
nondisjunction
Bread wheat
(Triticum aestivum)
Genome AA BB DD
(6n = 42)

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8.1 Modern bread wheat, *Triticum aestivum*, is a hexaploid with genes derived from three different species. Two diploid species, *T. urartu* ($2n = 14$) and probably *Aegilops speltoides* or a related species ($2n = 14$), originally crossed to produce a diploid hybrid ($2n = 14$) that underwent chromosome doubling to create *T. turgidum* ($4n = 28$). A cross between *T. turgidum* and *A. tauschii* ($2n = 14$) produced a triploid hybrid ($3n = 21$) that then underwent chromosome doubling to eventually produce *T. aestivum*, which is a hexaploid ($6n = 42$).

Description
In the P generation at the top, Einkorn wheat (Triticum uratu) is crossed with the wild grass (Aegilops speltoides or related species). Einkorn wheat has genome big A big A (2 n equals 14) and the wild grass has genome big B big B (2 n equals 14). The gametes from these 2 plants fuse and an arrow points to the F subscript 1 generation hybrid below with genome big A big B (2 n equals 14). An arrow labeled mitotic nondisjunction points from the hybrid to a picture of the Emmer wheat (Triticum turgidum) with genome big A big A big B big B (4 n equals 28) crossed with wild grass (Aegilops tauschii) having genome big D big D (2 n equals 14). The gametes from these two plants fuse and form the F subscript 2 generation hybrid below with genome big A big B big D (3 n equals 21). An arrow labeled mitotic nondisjunction points from the hybrid to a picture of the bread wheat (Triticum aestivum) having genome big A big A big B big B big D big D (6 n equals 42).

Some 10,000 years ago, two diploid grasses crossed, producing a hybrid with 14 chromosomes. The chromosomes of this hybrid then doubled, creating Emmer wheat (*Triticum turgidum*) with 28 chromosomes, which was domesticated by early farmers and is still cultivated in some parts of the world. Several thousand years later, Emmer wheat crossed with a third diploid species (Figure 8.1), giving rise to a new hybrid with 21 chromosomes. In the final step, this hybrid underwent yet another mitotic nondisjunction, doubling the

chromosomes to produce modern wheat (*Triticum aestivum*) with 42 chromosomes.

Over the ages, wheat has been subjected to selective breeding and genetic manipulation. Genetic modification of wheat in recent years, however, has been limited by the absence of detailed information about its genetic makeup. In 2005, a group of wheat farmers in Kansas saw the potential benefits of more genetic information and initiated an effort to sequence the wheat genome. This led to a large effort known as the International Wheat Genome Sequencing Consortium.

Sequencing the genome of wheat turned out to be a huge task. Wheat has a large genome, consisting of about 17 billion base pairs of DNA, compared to the human genome, with 3.2 billion base pairs. In addition, 85% of wheat's genome is made up of short, repeated DNA sequences, which are difficult to assemble into a full genome. Wheat's genome sequencing ultimately required 13 years to complete and the efforts of more than 200 scientists from 20 countries. The entire wheat genome was finally sequenced in 2017, and a fully annotated and assembled genome was published in 2018. The researchers identified 107,891 protein-encoding genes, over five times as many as in humans.

The completed genome sequence of wheat will allow researchers and plant breeders to more rapidly identify key genes involved in yield, nutrition, disease resistance, and stress tolerance. It will

undoubtedly aid in the breeding of new varieties that can be used to help feed the world's growing population. Already, researchers have used the genome sequence to find new genes that affect grain size, plant architecture, and the length of the wheat breeding cycle. Other researchers have identified a number of genes for proteins that cause immune responses in humans, which may help in the creation of new wheat varieties that are less allergenic.

THINK-PAIR-SHARE

- Modern wheat's genome consist of six sets of chromosomes derived from three different species. Can you think of any advantage to having a genome with genetic information from multiple species?

Most species have a characteristic number of chromosomes, each with a distinct size and structure, and all the tissues of an organism (except for gametes) generally have the same set of chromosomes. Nevertheless, variations in chromosome number—such as the extra sets of chromosomes seen in wheat—do periodically arise. Variations may also arise in chromosome structure: individual chromosomes may lose or gain parts, and the order of genes within a chromosome may be altered. These variations in the number and structure of chromosomes are termed **chromosome mutations**, and they frequently play an important role in agriculture and evolution.

We begin this chapter by reviewing some of the basic concepts of chromosome structure. We then consider the different types of

chromosome mutations and their features, phenotypic effects, and influences on evolution.

8.1 Chromosome Mutations Include Rearrangements, Aneuploidy, and Polyploidy

Before we consider the different types of chromosome mutations, their effects, and how they arise, it may be helpful to review and expand on the basics of chromosome structure that we learned in Chapter 2.

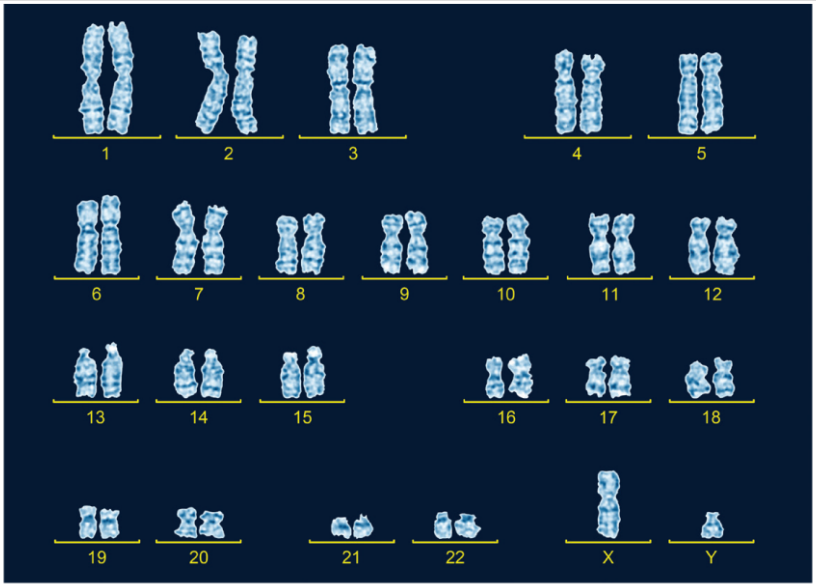
Chromosome Morphology

Each functional eukaryotic chromosome has a centromere, to which spindle microtubules attach, and two telomeres, which stabilize the chromosome ends (see Figure 2.7). Chromosomes are classified into four basic types:

1. **Metacentric**. The centromere is located approximately in the middle, so the chromosome has two arms of equal length.
2. **Submetacentric**. The centromere is displaced toward one end, creating a long arm and a short arm. (On human chromosomes, the short arm is designated by the letter *p* and the long arm by the letter *q*.)
3. **Acrocentric**. The centromere is near one end, producing a long arm and a knob, or satellite, at the other end.

4. **Telocentric**. The centromere is at or very near the end of the chromosome (see Figure 2.8).

The complete set of chromosomes possessed by an organism is called its *karyotype*. An organism's karyotype is usually presented as a picture of metaphase chromosomes lined up in descending order of their size (**Figure 8.2**). Karyotypes are prepared from actively dividing cells, such as white blood cells, bone-marrow cells, or cells from meristematic tissues of plants. After treatment with a chemical (such as colchicine) that prevents them from entering anaphase, the cells are chemically preserved. They are then burst open to release the chromosomes onto a microscope slide, and the chromosomes are stained and photographed. The photograph is then enlarged, and the individual chromosomes are cut out and arranged in a karyotype. For human chromosomes, karyotypes are routinely prepared by automated machines, which scan a slide using a video camera attached to a microscope, looking for a chromosome spread (a group of chromosomes that are well separated). When a spread has been located, the camera takes a picture of the chromosomes, the image is digitized, and the chromosomes are sorted and arranged electronically by a computer.



ISM/Pr. Philippe Vago/Medical Images.

8.2 A human karyotype consists of 46 chromosomes.

Description

Each chromosome pair is shown and its number is labeled below. The twenty third pair has 1 larger X chromosome and 1 relatively smaller Y chromosome.

Preparation and staining techniques help to distinguish among chromosomes of similar size and shape. For instance, special preparation and staining of chromosomes with a dye called Giemsa reveals G bands, which distinguish areas of DNA that are rich in adenine–thymine (A–T) base pairs (**Figure 8.3**; see also Chapter 10). Q bands are revealed by staining chromosomes with quinacrine mustard and viewing the chromosomes under ultraviolet light; variation in the brightness of Q bands results from differences in the relative numbers of cytosine–guanine (C–G) and adenine–thymine base pairs. Other techniques reveal C bands, which are regions of

DNA occupied by centromeric heterochromatin, and R bands, which are rich in cytosine–guanine base pairs.

Leonard Lessin/Science Source.

8.3 Chromosome banding is revealed by special staining techniques. These chromosomes have undergone Giemsa staining to produce G banding.

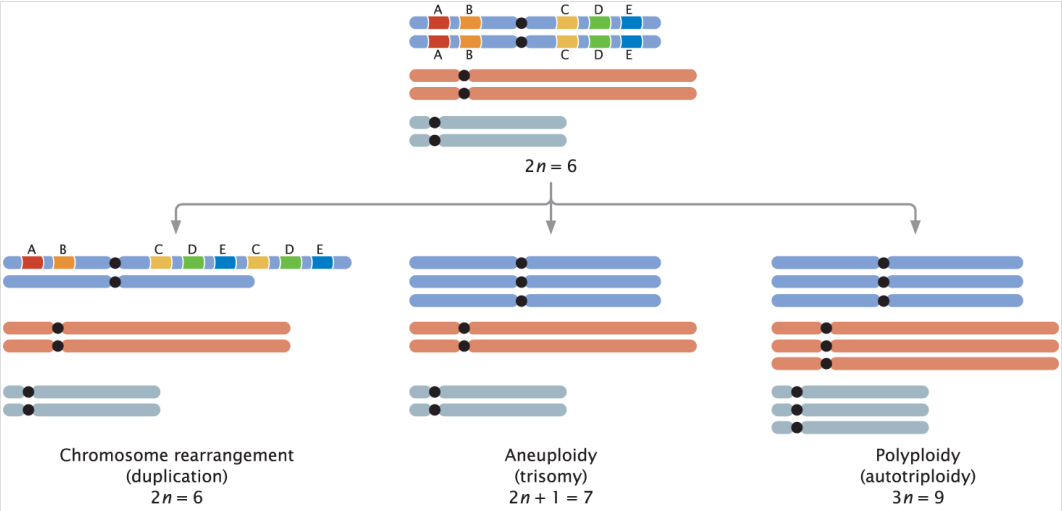
Description
This karyotype shows the chromosomes lined up and clustered in groups. The chromosomes have several bands of light gray.

Types of Chromosome Mutations

Chromosome mutations can be grouped into three basic categories: chromosome rearrangements, aneuploidy, and polyploidy (Figure 8.4). Chromosome rearrangements alter the *structure* of

chromosomes: for example, a piece of a chromosome may be duplicated, deleted, or inverted. In aneuploidy, the *number* of chromosomes is altered: one or more individual chromosomes are added or deleted. In polyploidy, one or more complete *sets* of chromosomes are added. A polyploid is any organism that has more than two sets of chromosomes (3*n*, 4*n*, 5*n*, or more).

THINK-PAIR-SHARE Question 1



8.4 The three basic categories of chromosome mutations are chromosome rearrangements, aneuploidy, and polyploidy. Duplication, trisomy, and autotriploidy, respectively, are examples of these categories of mutations.

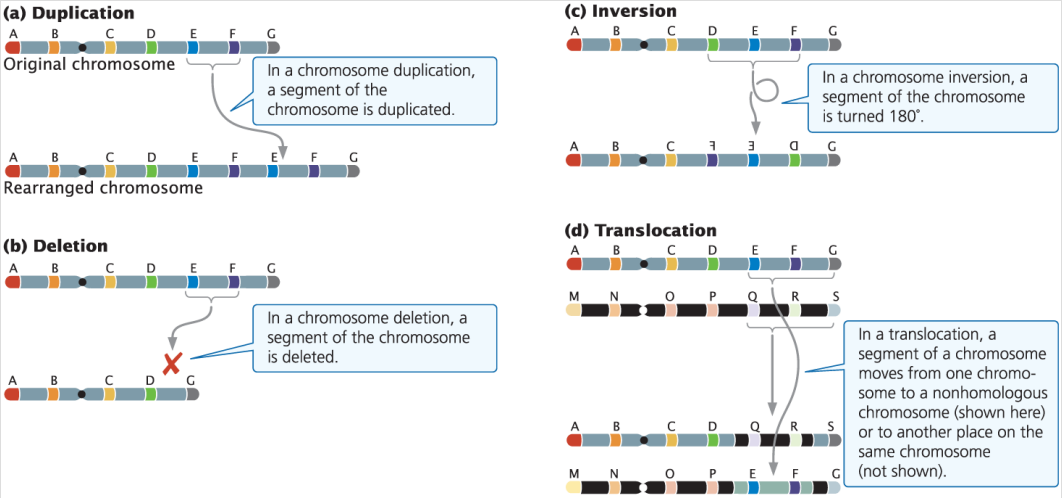
Description

The diagram shows 3 sets of chromosomes. The first has genes big A and big B on both the chromosomes above the centromere and big C, big D, and big E on both the chromosomes below the centromere. Second set of chromosomes has longer arm below the centromere and the third set also has the longer arm below the centromere but is relatively shorter (as compared to the second set). A text below reads, 2 n equals 6.

Chromosome rearrangement (duplication) occurs at left below and the first set of chromosome gets the genes big C, big D, and big E duplicated on one of its chromosomes. The chromosome set now has genes big and big B on one chromosome above the centromere and big C, big D, big E, big C, big D, and big E on that chromosome below the centromere. The long arm of the chromosome with these genes is relatively longer than the other chromosome in the set. The other two sets of chromosomes remain the same as original. A text below reads, 2 n equals 6. Aneuploidy (trisomy) occurs at center below and the first set of chromosome gets one extra chromosome while other 2 sets remain same as original. A text below reads, 2 n plus 1 equals 7. Polyploidy (Autotriploid) occurs at right below and each set gets one similar extra chromosome. A text below reads, 3 n equals 9.

8.2 Chromosome Rearrangements Alter Chromosome Structure

Chromosome rearrangements are chromosome mutations that change the structure of individual chromosomes. The four basic types of rearrangements are duplications, deletions, inversions, and translocations (**Figure 8.5**). Many of these chromosome rearrangements originate when double-stranded breaks occur in the DNA molecule found within a chromosome. Double-stranded breaks in DNA often cause cell death, so organisms have evolved elaborate mechanisms to repair breaks by reconnecting the broken ends of DNA (see Repair of Double-Strand Breaks in Chapter 18). If the two broken ends are rejoined correctly, the original chromosome is restored, and no chromosome rearrangement results. Sometimes the wrong ends are connected, however, leading to a chromosome rearrangement. Chromosome rearrangements can also arise through errors in crossing over or when crossing over occurs between repeated DNA sequences.



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8.5 The four basic types of chromosome rearrangements are duplications, deletions, inversions, and translocations.

Description

Each part begins with a chromosome containing genes big A through big G.

In the first part at the top left, the caption reads: In chromosome duplication, a segment of the chromosome is duplicated. In the picture, the genes big E and big F are duplicated. On the new strand, there is a second set of genes big E and big F between genes big F and big G.

In the second part at the bottom left, the caption reads: in chromosome deletion, a segment of the chromosome is deleted. In the picture, the genes e and f are deleted. On the new strand, there are only 5 genes: big A, big B, big C, big D, and big G.

In the third part at the top right, the caption reads: in chromosome inversion, a segment of the chromosome is turned 180 degrees. In the picture, genes big D, big E, and big F are flipped in alignment. On the new strand, there are genes big A, big B, big C, backwards big F, backwards big E, backwards big D, and big G.

The fourth part at the bottom right begins with an additional chromosome containing genes big M through big S, and the caption reads: In translocation, a segment of a chromosome moves from one chromosome to a non-homologous chromosome (shown here) or to another place on the same chromosome (not shown). In this picture, genes big E, big F, and big G on the one chromosome switch places with big Q, big R, and big S on the other.

Duplications

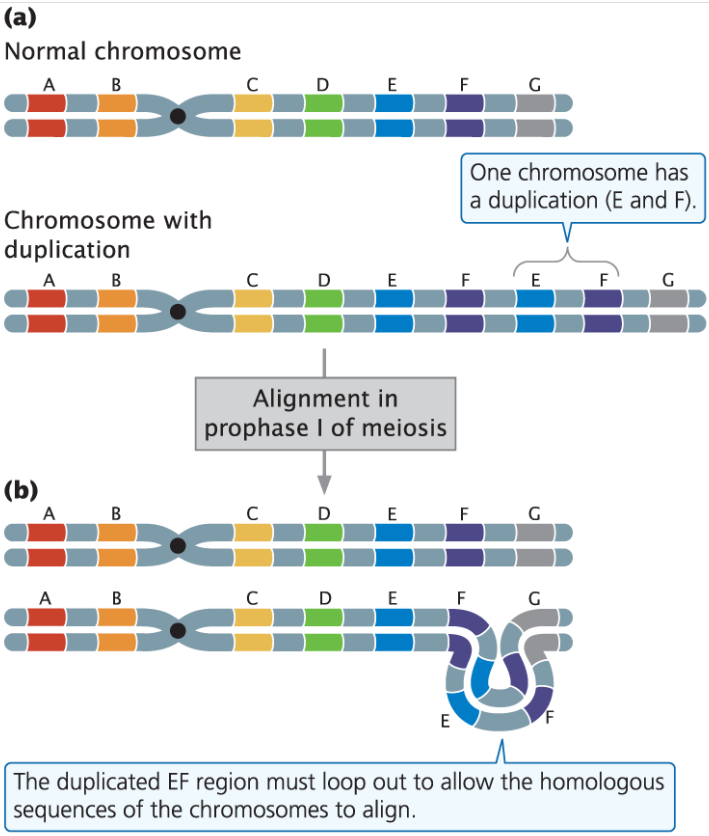
A **chromosome duplication** is a mutation in which part of the chromosome has been doubled (see Figure 8.5a). Consider a chromosome with segments AB•CDEFG, in which • represents the centromere. A duplication might include the EF segments, giving rise to a chromosome with segments AB•CDEFEFG (where the underlined letters represent the part of the chromosome that has changed). This type of duplication, in which the duplicated segment is immediately adjacent to the original segment, is called a **tandem duplication**. If the duplicated segment is located some distance from the original segment, either on the same chromosome or on a different one, the chromosome rearrangement is called a **displaced duplication**. An example of a displaced duplication would be AB•CDEFGEF. A duplication can have the same orientation as the original sequence, as in the two preceding examples, or it can be inverted: AB•CDEFFEG. When the duplication is inverted, it is called a **reverse duplication**.

The human genome contains numerous duplicated sequences called **segmental duplications**, which are defined as duplications greater than a thousand base pairs (bp) in length. Most segmental duplications are *intrachromosomal* duplications (i.e., the two copies are found on the same chromosome), but others are

interchromosomal duplications (the two copies are found on different chromosomes).

EFFECTS OF CHROMOSOME DUPLICATIONS

An individual that is homozygous for a duplication carries that duplication on both homologous chromosomes, and an individual that is heterozygous for a duplication has one normal chromosome and one chromosome with the duplication. In heterozygotes (**Figure 8.6a**), problems in chromosome pairing arise at prophase I of meiosis because the two chromosomes are not homologous throughout their length. The pairing and synapsis of homologous regions require that one or both chromosomes loop and twist so that these regions are able to line up (**Figure 8.6b**). The appearance of this characteristic loop structure in meiosis is one way to detect duplications.

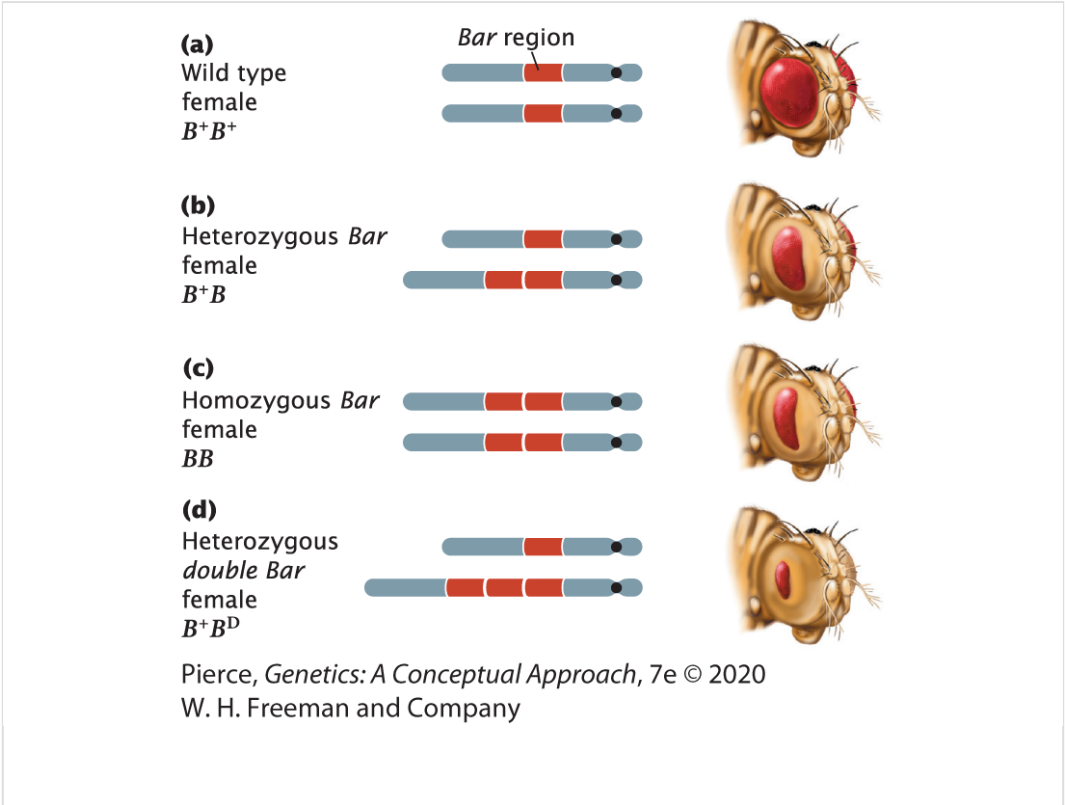


8.6 In an individual heterozygous for a duplication, the duplicated region loops out during pairing in prophase I.

Description

Part a at the top shows a normal chromosome with genes big A and big B on the short arms of both the chromatids and big C, big D, big E, big F, and big G on the long arms of both the chromatids. The chromosome with duplication has genes big A and big B on both the chromatids on the short arms and big C, big D, big E, big F, big E (duplicated gene), big F (duplicated gene), and big G on both the chromatids on the long arms. A caption reads, One chromosome has a duplication (big E and big F). Alignment occurs in prophase 1 of meiosis and leads to part b below which shows the chromosomes after alignment. The Normal chromosome has genes with the same alignment as part a. The chromosome below or the chromosome with duplication has the duplicated region looped out with genes big E big F. A caption pointing towards the looped out region reads, The duplicated big E big F region must loop out to allow the homologous sequences of the chromosomes to align.

Duplications may have major effects on the phenotype. Among fruit flies, for example, a fly with the *Bar* mutation has a reduced number of facets in the eye, making the eye smaller and bar shaped instead of oval (**Figure 8.7**). The *Bar* mutation results from a small duplication on the X chromosome that is inherited as an incompletely dominant, X-linked trait: heterozygous female flies have somewhat smaller eyes (the number of facets is reduced; see Figure 8.7b), whereas in homozygous female and hemizygous male flies, the number of facets is greatly reduced (see Figure 8.7c). Occasionally, a fly carries three copies of the *Bar* duplication on its X chromosome; in flies with this mutation, termed *double Bar*, the number of facets is extremely reduced (Figure 8.7d).



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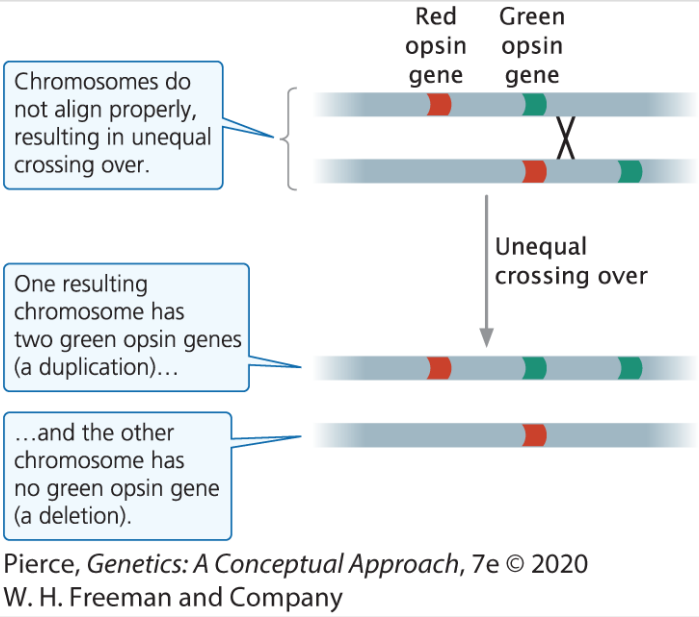
8.7 The Bar phenotype in *Drosophila melanogaster* results from an X-linked duplication. (a) Wild-type fruit flies have normal-sized eyes. (b) Flies that are heterozygous and (c) homozygous for the *Bar* mutation have smaller, bar-shaped eyes. (d) Flies with *double Bar* have three copies of the duplication and much smaller bar-shaped eyes.

Description
Part a at the top shows a wild type female fly with genes big B superscript positive big B superscript positive. Both the chromosomes contain 1 copy each of bar gene. The fly has large, round red-colored eyes. Part b below shows a heterozygous bar female with genes big B superscript positive big B. The top chromosome has 1 copy of bar gene and the bottom chromosome has 2 copies of bar gene. The fly has thick bar eyes. Part c below shows a homozygous bar female with genes big B big B. Both the chromosomes have 2 copies each of bar gene. The fly has relatively thinner bar eyes. Part d below shows a heterozygous double bar female with genes big B superscript positive big B superscript D. The top chromosome has 1 copy of bar gene and the bottom chromosome has 3 copies of bar gene. The fly has the thinnest and shortest bar eyes.

Duplications and deletions often arise from **unequal crossing over**, in which chromosomes misalign during crossing over. Unequal crossing over is frequently the cause of red–green color blindness in humans. Perception of color is affected by red and green opsin genes, which are found on the X chromosome and are 98% identical in their DNA sequence. Most people with normal color vision have one red opsin gene and one green opsin gene (although some people have more than one copy of each). Occasionally, two paired X chromosomes in a female do not align properly in prophase I, and unequal crossing over takes place. The unequal crossing over produces one chromosome with an extra opsin gene and one chromosome that is missing an opsin gene (**Figure 8.8**; see also

Figure 18.14). When a male inherits the chromosome that is missing one of the opsin genes, red–green color blindness results.



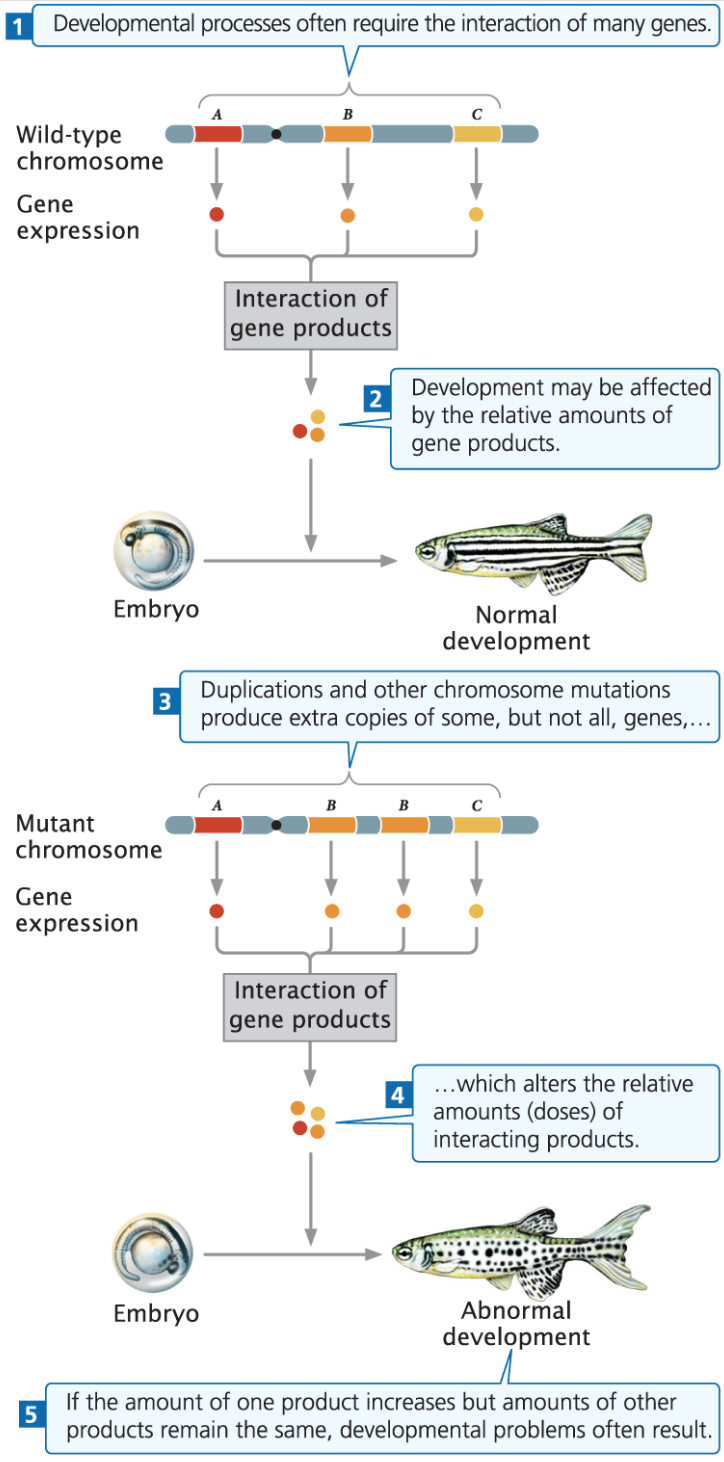
8.8 Unequal crossing over produces duplications and deletions.

Description

A chromosome has a red opsin gene and a green opsin gene. Below this chromosome, a chromosome has the same genes but its red gene is aligned below the green gene of the above chromosome. A caption reads, Chromosomes do not align properly, resulting in unequal crossing over. Unequal crossing over occurs and resulting chromosomes are obtained below. The resulting top chromosome has 1 red opsin gene and 2 green opsin genes (a duplication) and the bottom chromosome has only red opsin gene and green opsin gene is missing (a deletion). A caption reads, One resulting chromosome has two green opsin genes (a duplication) and the other chromosome has no green opsin gene (a deletion).

UNBALANCED GENE DOSAGE

How does chromosome duplication alter the phenotype? After all, gene sequences are not altered by duplications, and no genetic information is missing; the only change is the presence of additional copies of normal sequences. The answer to this question is not well understood, but the effects are most likely due to imbalances in the amounts of gene products (abnormal gene dosage). The amount of a particular protein synthesized by a cell is often directly related to the number of copies of its corresponding gene: an individual organism with three functional copies of a gene often produces 1.5 times as much of the protein encoded by that gene as an individual with two copies. Because developmental processes require the interaction of many proteins, they often depend critically on proper gene dosage. If the amount of one protein increases while the amounts of others remain constant, problems can result (**Figure 8.9**). Duplications can have severe consequences when the precise balance of gene products is critical to cell function (**Table 8.1**).



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8.9 Unbalanced gene dosage leads to developmental abnormalities.

Description
The diagram shows a wild-type chromosome with genes big A, big B, and big C at the top. A caption reads, Developmental processes often require the interaction of many genes. Below the chromosome, the individual genes are expressed and the gene products interact. A caption reads, Development may be affected by the relative amounts of gene products. An embryo below is influenced by the interaction of gene products which leads to normal development of a fish. This fish has stripes running horizontally over the body.

A mutant chromosome below shows genes big A, big B, big B, and big C. A caption reads, Duplications and other chromosome mutations produce extra copies of some, but not all, genes, which alters the relative amounts (doses) of interacting products. The individual genes are expressed and the gene products interact. An embryo below is influenced by the interaction of gene products which leads to abnormal development of a fish. It has a different appearance than the normal fish and has spots rather than stripes. A caption below reads, If the amount of one product increases but amount of other products remain the same, developmental problems often result.

TABLE 8.1 Effects of some human chromosome rearrangements

Type of Rearrangement	Chromosome	Disorder	Symptoms
Duplication	4, short arm	—	Small head, short neck, low hairline, reduced growth, intellectual disability
Duplication	4, long arm	—	Small head, sloping forehead, hand abnormalities
Duplication	7, long arm	—	Delayed development, asymmetry of the head, fuzzy scalp, small nose, low-set ears
Duplication	9, short arm	—	Characteristic facial features, variable intellectual disability, high and broad forehead, hand abnormalities
Deletion	5, short arm	*Cri-du-chat* syndrome	Small head, distinctive cry, widely spaced eyes, round face, intellectual disability
Deletion	4, short arm	Wolf–	Small head with high forehead, wide nose,

		Hirschhorn syndrome	cleft lip and palate, severe intellectual disability
Deletion	4, long arm	—	Small head, mild to moderate intellectual disability, cleft lip and palate, hand and foot abnormalities
Deletion	7, long arm	Williams–Beuren syndrome	Distinctive facial features, heart defects, cognitive impairment
Deletion	15, long arm	Prader–Willi syndrome	Feeding difficulty at early age but becoming obese after one year of age, mild to moderate intellectual disability
Deletion	18, short arm	—	Round face, large and low-set ears, mild to moderate intellectual disability
Deletion	18, long arm	—	Distinctive mouth shape, small hands, small head, intellectual disability

IMPORTANCE OF DUPLICATIONS IN EVOLUTION

Duplications have arisen frequently throughout the evolution of many eukaryotic organisms. Chromosome duplications are one way in which new genes evolve. In many cases, existing copies of a gene are not free to vary because they encode a product that is essential to development or function. However, after a chromosome undergoes duplication, extra copies of genes within the duplicated region are present. The original copy can provide the essential function, whereas an extra copy from the duplication is free to undergo mutation and change. Over evolutionary time, the extra copy may acquire enough mutations to assume a new function that benefits the organism. For example, humans have a series of genes that encode different globin chains, which function as oxygen

carriers. Some of these globin chains function during adult stages, and others function during embryonic and fetal development. The genes encoding all of these globins arose from an original primordial globin gene that underwent a series of duplications (see Figure 26.19). Segmental duplications have played an important role in the evolution of human chromosomes; about 4% of the human genome consists of segmental duplications.

CONCEPTS

A chromosome duplication is a mutation that doubles part of a chromosome. In individuals heterozygous for a chromosome duplication, the duplicated region of the chromosome loops out when homologous chromosomes pair in prophase I of meiosis. Duplications often have major effects on the phenotype, possibly by altering gene dosage. Segmental duplications are common within the human genome.

✔ CONCEPT CHECK 1

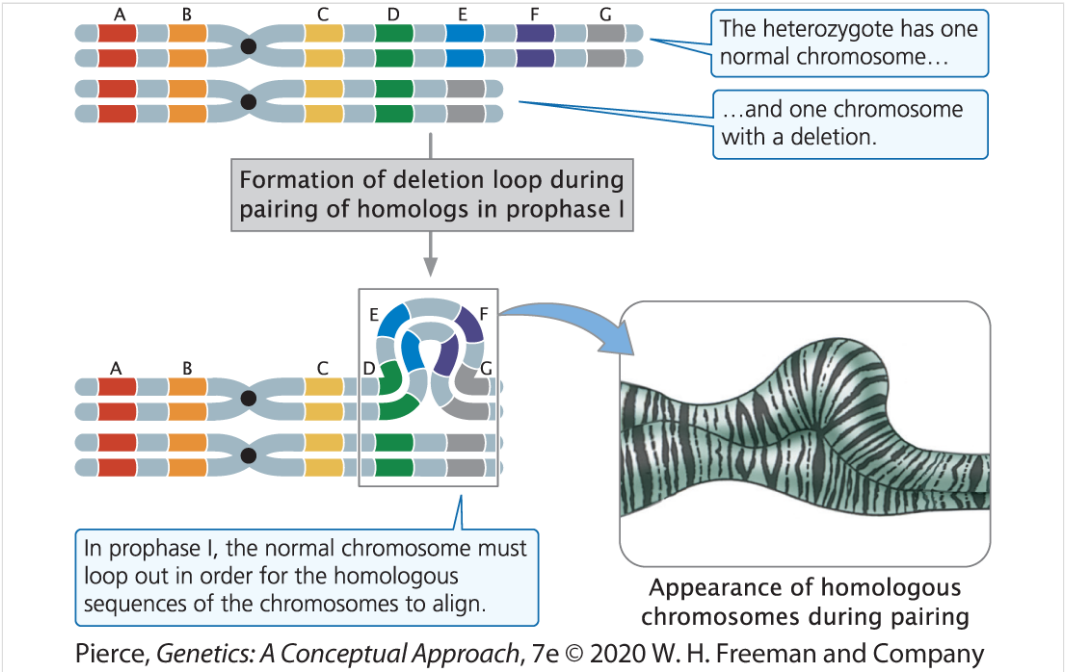
Chromosome duplications often result in abnormal phenotypes because

a. developmental processes depend on the relative amounts of proteins encoded by different genes.
b. extra copies of the genes within the duplicated region do not pair in meiosis.
c. the chromosome is more likely to break when it loops in meiosis.
d. extra DNA must be replicated, which slows down cell division.

Deletions

A second type of chromosome rearrangement is a **chromosome deletion**: the loss of a chromosome segment (see Figure 8.5b). A chromosome with segments AB•CDEFG that undergoes a deletion of segment EF would generate the mutated chromosome AB•CDG.

A large deletion can be easily detected because the chromosome is noticeably shortened. In individuals heterozygous for deletions, the normal chromosome must loop out during the pairing of homologs in prophase I of meiosis (**Figure 8.10**) to allow the homologous regions of the two chromosomes to align and undergo synapsis. This looping out generates a structure that looks very much like that seen in individuals heterozygous for duplications.



8.10 In an individual heterozygous for a deletion, the normal chromosome loops out during chromosome pairing in prophase I.

Description
The diagram shows 2 homologous chromosomes. The top chromosome has genes big A, big B, big C, big D, big E, big F, and big G. The bottom chromosome has genes big A, big B, big C, big D, and big G. A caption reads, The heterozygote has one normal chromosome and one chromosome with a deletion. Formation of deletion loop during pairing of homologs in prophase 1 occurs. The resultant chromosmes are shown below. Both the homologous chromosomes are aligned but the genes big E and big F are looping out from the top chromosome. A caption reads, In prophase 1, the normal chromosome must loop out in order for the homologous sequences of the chromosomes to align. The illustration also shows a close-up of homologous chromosomes during pairing where the top chromosome is looped out from a region. The other regions are aligned and vertical stripes are present all over the aligned chromosomes.

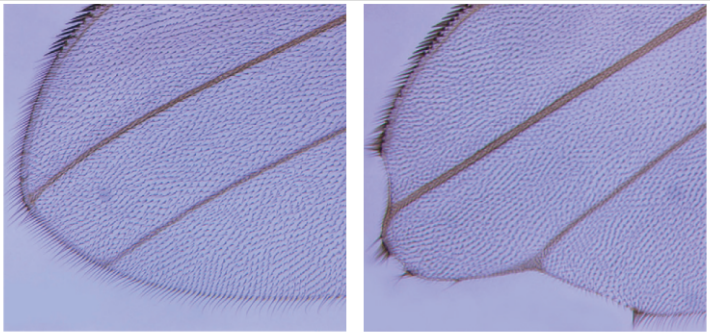
EFFECTS OF DELETIONS

The phenotypic consequences of a deletion depend on which genes are located in the deleted region. If the deletion includes the centromere, then the chromosome will not segregate in meiosis or mitosis and will usually be lost. Many deletions are lethal in the homozygous state because all copies of any essential genes located in the deleted region are missing.

Even individuals heterozygous for a deletion may have multiple defects for three reasons. First, the heterozygous condition may produce imbalances in the amounts of gene products similar to those produced by extra gene copies. Second, normally recessive mutations on the homologous chromosome lacking the deletion may be expressed when the wild-type allele has been deleted (and is no longer present to mask the recessive allele's expression). The

expression of a normally recessive mutation is referred to as **pseudodominance**, and it is an indication that one of the homologous chromosomes has a deletion.

Third, some genes must be present in two copies for normal function. When a single copy of a gene is not sufficient to produce a wild-type phenotype, that gene is said to be **haploinsufficient**. A series of X-linked wing mutations in *Drosophila* are known as *Notch* mutations. These mutations often result from chromosome deletions. *Notch* deletions behave in a dominant manner: when heterozygous for a *Notch* deletion, a fly has wings that are notched at the tips and along the edges (**Figure 8.11**). The *Notch* gene is therefore haploinsufficient. Females that are homozygous for a *Notch* deletion (or males that are hemizygous) die early in embryonic development. The *Notch* gene, which is deleted in *Notch* mutants, encodes a receptor that normally transmits signals received from outside the cell to the cell's interior and is important in fly development. The deletion acts as a recessive lethal because the loss of all copies of the *Notch* gene prevents normal development.



Spyros Artavanis-Tsakonas, Kenji Matsuno, and Mark E. Fortini.

8.11 The Notch phenotype is produced by a chromosome deletion that includes the *Notch* gene. (Left) Normal wing venation. (Right) Wing venation produced by a *Notch* mutation.

Description
The photo on the left shows a normal wing venation with a round outer edge. The photo on the rights shows wing venation with notches at the edges and tips in the wing as a result of Notch mutation.

CHROMOSOME DELETIONS IN HUMANS

In humans, a deletion on the short arm of chromosome 5 is responsible for *cri-du-chat* syndrome. The name (French for "cry of the cat") derives from the peculiar, catlike cry of infants with this syndrome. A child who is heterozygous for this deletion has a small head, widely spaced eyes, and a round face, and is intellectually disabled. Deletion of part of the short arm of chromosome 4 results in another human disorder, Wolf–Hirschhorn syndrome, which is characterized by seizures, severe intellectual disability, and delayed growth. A deletion of a tiny segment of chromosome 7 causes haploinsufficiency of the gene encoding elastin and a few other genes and leads to a condition known as Williams–Beuren syndrome, which is characterized by distinctive facial features, heart defects, high blood pressure, and cognitive impairments. The effects of some deletions in human chromosomes are summarized in Table 8.1.

CONCEPTS

A chromosome deletion is a mutation in which a part of a chromosome is lost. In individuals heterozygous for a deletion, the normal chromosome loops out during prophase I of meiosis. Deletions cause recessive genes on the homologous chromosome to be expressed and may cause imbalances in gene products.

✔ CONCEPT CHECK 2

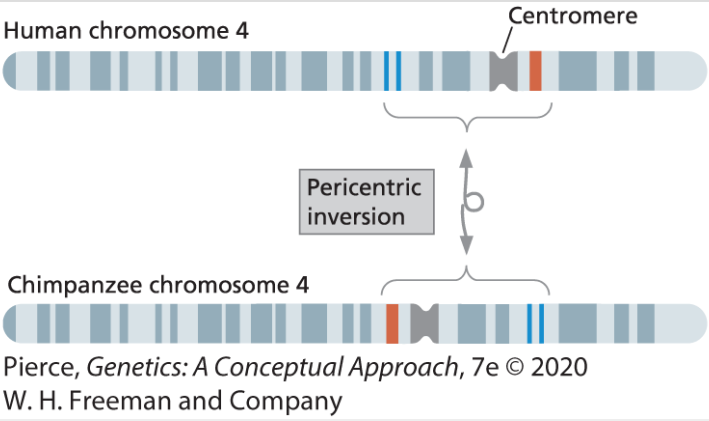
What is pseudodominance, and how is it produced by a chromosome deletion?

Inversions

A third type of chromosome rearrangement is a **chromosome inversion**, in which a chromosome segment is inverted—turned 180 degrees (see Figure 8.5c). If a chromosome originally had segments AB•CDEFG, then chromosome AB•CFEDG represents an inversion that includes segments DEF. For an inversion to take place, the chromosome must break in two places. Inversions that do not include the centromere, such as AB•CFEDG, are termed **paracentric inversions** (*para* meaning "next to"), whereas inversions that include the centromere, such as ADC•BEFG, are termed **pericentric inversions** (*peri* meaning "around").

Inversion heterozygotes are common in many organisms, including a number of plants, some species of *Drosophila*, mosquitoes, and

grasshoppers. Inversions may have played an important role in human evolution: G-banding patterns reveal that several human chromosomes differ from those of chimpanzees by only a pericentric inversion (Figure 8.12).



8.12 Chromosome 4 in humans and in chimpanzees differs by a pericentric inversion.

Description
Human chromosome 4 shown at the top has same genes as that in chimpanzee chromosome 4 shown below, except a small region around centromere that is inversed in the chimpanzee chromosome 4. This inversion is marked as pericentric inversion.

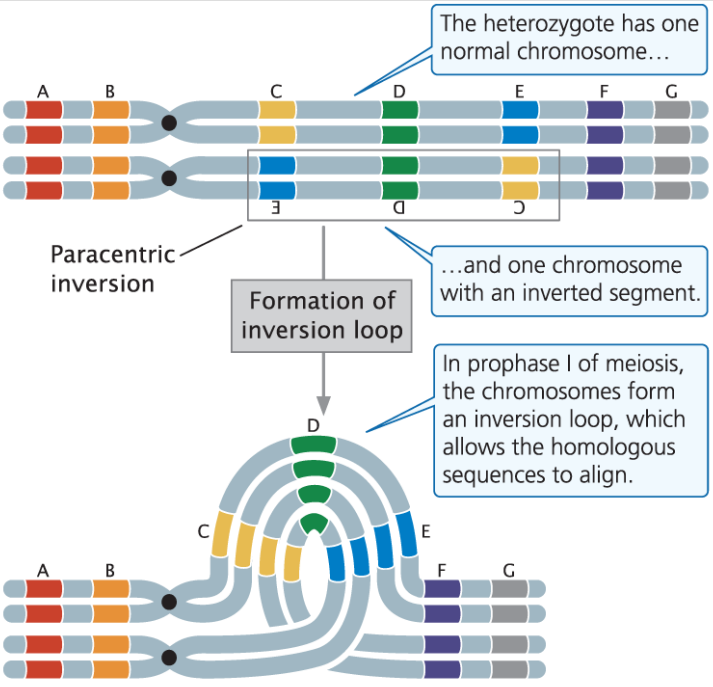
EFFECTS OF INVERSIONS

Individual organisms with inversions have neither lost nor gained any genetic material; only the DNA sequence has been altered. Nevertheless, these mutations often have pronounced phenotypic effects. An inversion may break a gene into two parts, with one part moving to a new location and destroying the function of that gene.

Even when the chromosome breaks lie between genes, phenotypic effects may arise from the inverted gene order. Many genes are regulated in a position-dependent manner; if their positions are altered by an inversion, their expression may be altered, an outcome referred to as a **position effect**. For example, when an inversion moves a wild-type allele (which normally encodes red eyes) at the *white* locus in *Drosophila* to a chromosomal region that contains highly condensed and inactive chromatin, the wild-type allele is not expressed in some cells, resulting in an eye consisting of red and white spots.

INVERSIONS IN MEIOSIS

When an individual is homozygous for a particular inversion, no special problems arise in meiosis, and the two homologous chromosomes can pair and separate normally. However, when an individual is heterozygous for an inversion, the gene order of the two homologs differs, and the homologous sequences can align and pair only if the two chromosomes form an inversion loop (**Figure 8.13**).



8.13 In an individual heterozygous for a paracentric inversion, the chromosomes form an inversion loop during pairing in prophase I.

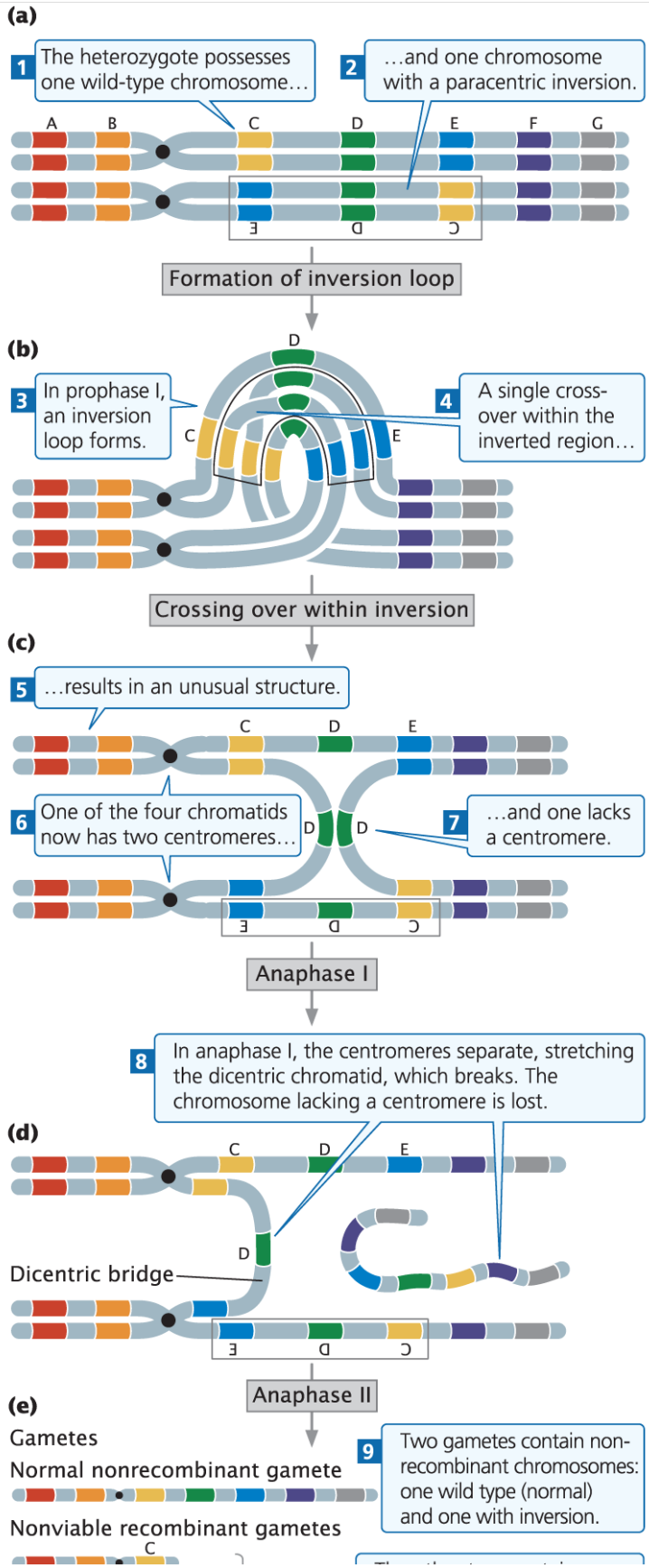
Description

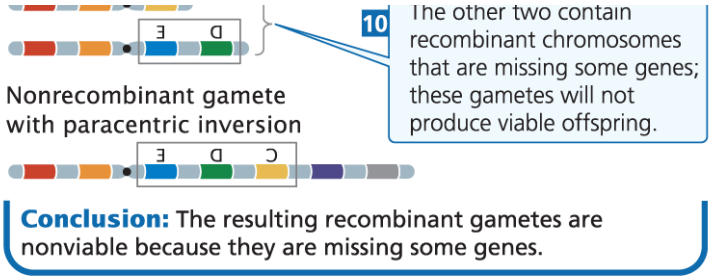
The diagram shows a pair of homologous chromosomes. The top chromosome has the genes big A, big B, big C, big D, big E, big F, and big G. The bottom chromosome has paracentric inversion involving genes big C, big D, and big E that are highlighted in a box. A caption reads, The heterozygote has one normal chromosome and one chromosome with an inverted segment. Formation of inversion loop occurs and the resultant chromosomes are shown below. The top chromosome forms a bump involving genes big C, big D, and big E, while the bottom chromosome rises upwards, turns down and eventually lies parallel to the top chromosome. Genes big C, big D, and big E on both the chromosomes are aligned in the loop. A caption reads, In prophase 1 of meiosis, the chromosomes form an inversion loop, which allows the homologous sequences to align.

Individuals heterozygous for inversions also exhibit reduced recombination among genes located in the inverted region. When

crossing over takes place, the outcome is abnormal gametes that do not give rise to viable offspring, and thus no recombinant progeny are observed. Let's see why this happens.

Figure 8.14 illustrates the results of crossing over within a paracentric inversion. Suppose that an individual is heterozygous for an inversion (see Figure 8.14a), with one wild-type, nonmutated chromosome (AB•CDEFG) and one inverted chromosome (AB•EDCFG). In prophase I of meiosis, an inversion loop forms, allowing the homologous sequences to pair up (see Figure 8.14b). If a single crossover takes place in the inverted region (between segments C and D in Figure 8.14), an unusual structure results (see Figure 8.14c). The two outer chromatids, which did not participate in crossing over, contain original, nonrecombinant gene sequences. The two inner chromatids, which did participate in crossing over, are highly abnormal: each has two copies of some genes and no copies of others. Furthermore, one of the four chromatids now has two centromeres, and is therefore referred to as a **dicentric chromatid**; the other lacks a centromere and is an **acentric chromatid**.


(a)
1 The heterozygote possesses one wild-type chromosome…
2 …and one chromosome with a paracentric inversion.
A B C D E F G
Formation of inversion loop
(b)
3 In prophase I, an inversion loop forms.
4 A single cross-over within the inverted region…
Crossing over within inversion
(c)
5 …results in an unusual structure.
6 One of the four chromatids now has two centromeres…
7 …and one lacks a centromere.
Anaphase I
8 In anaphase I, the centromeres separate, stretching the dicentric chromatid, which breaks. The chromosome lacking a centromere is lost.
(d)
Dicentric bridge
Anaphase II
(e)
Gametes
Normal nonrecombinant gamete
9 Two gametes contain non-recombinant chromosomes: one wild type (normal) and one with inversion.
Nonviable recombinant gametes



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8.14 In a heterozygous individual, a single crossover within a paracentric inversion leads to abnormal gametes.

Description

Part a at the top shows 2 homologous chromosomes. The first chromosome has genes big A, big B, big C, big D, big E, big F, and big G and the bottom chromosome has the same genes with an inverted portion containing big C, big D, big E. A caption reads, The heterozygote possesses one wild type chromosome and one with a paracentric inversion.

Part b below shows the chromosomes aligned and forming an inversion loop with genes big C, big D, and big E. A caption reads, In prophase 1, an inversion loop forms and a single crossover within the inverted region results in an unusual structure. Crossing over occurs within the inversion. The crossing over is outlined in a semi-circular shaped box.

Part c below shows the results of a crossover at the inversion. The two X-shaped chromosomes, instead of being separated, are connected at one of their arms involving gene big D. So, these X-shaped chromosomes are now fused. Also, the remaining part of the arm where the crossover occurred is now fused, but a chromosome has no centromere. A caption reads, One of the four chromatids now has two centromeres, and one lacks a centromere. Genes big C, big D, and big E on the bottom chromosome are outlined in a rectangular box.

Part d below shows the fused chromosome and the chromatid without the centromere. A dicentric bridge between the arms that are fused is formed. This bridge has gene big D. The chromatid without centromere is separated now. A caption reads, In anaphase 1, the centromeres separate, stretching the dicentric chromatid, which breaks. The chromosome lacking a centromere is lost. Genes big C, big D, and big E on the bottom chromosome are outlined in a rectangular box. Anaphase 2 occurs.

Part e below shows the resulting gametes. A caption reads, Two gametes contain non-recombinant chromosomes: one wild type (normal) and one with the inversion. The 2 normal, nonviable recombinant gametes are shorter and do not contain all of the genes present in the original. One gamete here has big C gene and other gamete has big D and big E gene which are highlighted in a box. A caption reads, The other two contain recombinant chromosomes that are missing some genes; these gametes will not produce viable offspring. A normal non recombinant gamete has all the genes that are in original and one Non recombinant gamete has all the genes involved in the paracentric inversion; big C, big D, and big E. These genes are highlighted in a rectangular box.

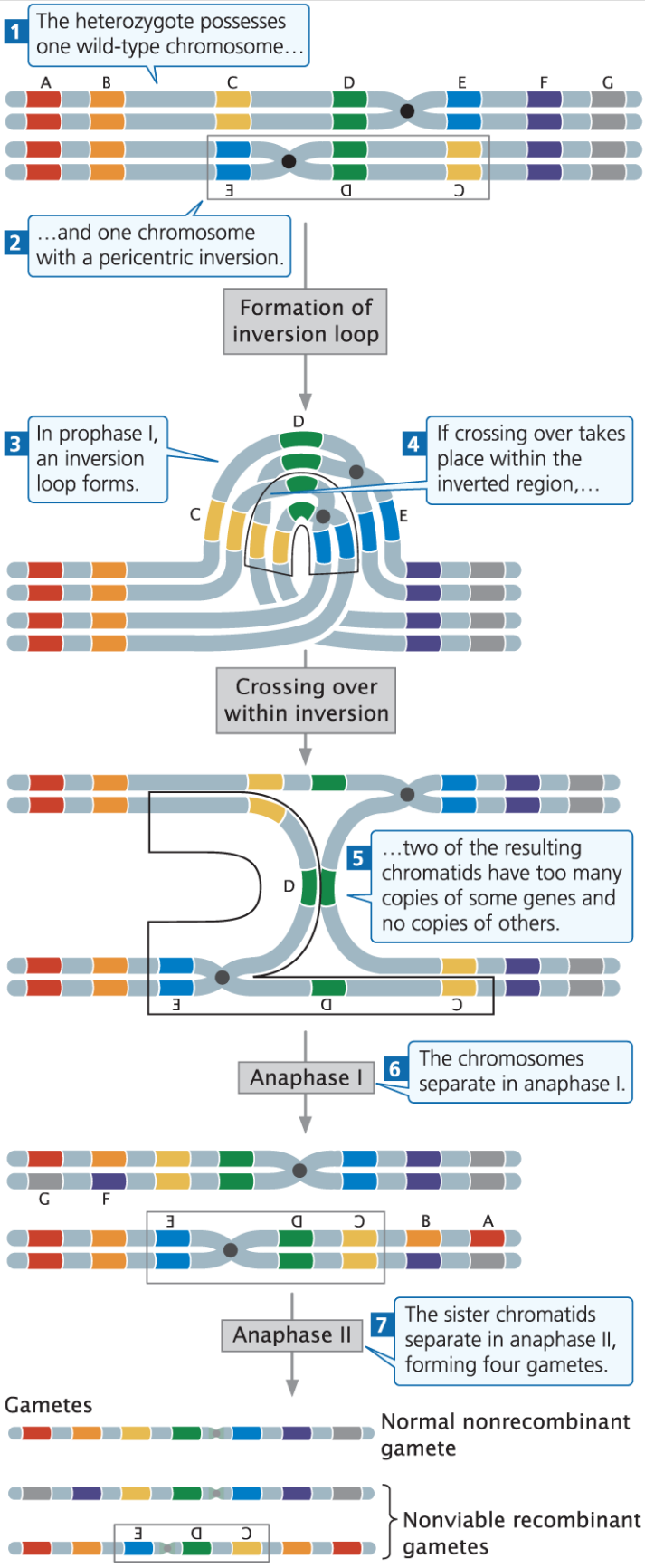
A caption below reads, Conclusion: The resulting recombinant gametes are nonviable because they are missing some genes.

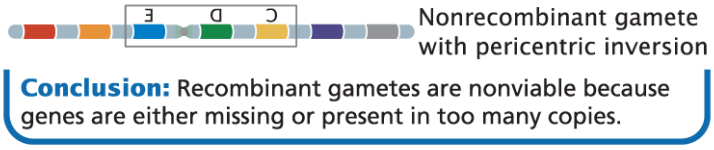
In anaphase I of meiosis, the centromeres are pulled toward opposite poles, and the two homologous chromosomes separate. This action stretches the dicentric chromatid across the center of the nucleus, forming a structure called a **dicentric bridge** (see Figure 8.14d). Eventually, the dicentric bridge breaks as the two centromeres are pulled farther apart. Spindle microtubules do not attach to the acentric fragment, so that fragment does not segregate to a spindle pole and is usually lost when the nucleus re-forms.

In the second division of meiosis, the sister chromatids separate, and four gametes are produced (see Figure 8.14e). Two of the gametes contain the original, nonrecombinant chromosomes (AB•CDEFG and AB•EDCFG). The other two gametes contain recombinant chromosomes that are missing some genes; these gametes will not produce viable offspring. Thus, no recombinant progeny result when crossing over takes place within a paracentric inversion. The key is to recognize that when crossing over takes

place, the resulting recombinant gametes are not viable, so no recombinant progeny are observed.

Recombination is also reduced within a pericentric inversion (**Figure 8.15**). No dicentric bridges or acentric fragments are produced, but the recombinant chromosomes have too many copies of some genes and no copies of others, so gametes that receive the recombinant chromosomes cannot produce viable progeny.


1 The heterozygote possesses one wild-type chromosome…
A B C D E F G
2 …and one chromosome with a pericentric inversion.
Formation of inversion loop
3 In prophase I, an inversion loop forms.
4 If crossing over takes place within the inverted region,…
Crossing over within inversion
5 …two of the resulting chromatids have too many copies of some genes and no copies of others.
Anaphase I
6 The chromosomes separate in anaphase I.
Anaphase II
7 The sister chromatids separate in anaphase II, forming four gametes.
Gametes
Normal nonrecombinant gamete
Nonviable recombinant gametes



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8.15 In a heterozygous individual, a single crossover within a pericentric inversion leads to abnormal gametes.

Description

The diagram shows a pair of chromosomes at the top. The top chromosome has genes big A, big B, big C, big D, big E, big F, and big G and the bottom chromosome has the same genes with pericentric inversion of genes big C, big D, big E. A caption reads, The heterozygote possesses one wild-type chromosome and one chromosome with a pericentric inversion. Formation of inversion loop involving genes big C, big D, and big E occurs below. The genes are highlighted in a semi-circular box to indicate crossing over. A caption reads, In prophase 1, an inversion loop forms. Another caption reads, If crossing over takes place within the inverted region, two of the resulting chromatids have too many copies of some genes and no copies of others. Crossing over within inversion occurs below. The two X-shaped chromosomes, instead of being separated, are connected at one of their arms involving gene big D, fusing the X-shaped chromosomes. Also, the remaining part of the arm where the crossover occurred is now fused. Genes big C, big D, and big E on the bottom chromosome and big G genes involved in fusing are outlined in a box. Anaphase 1 occurs and a caption reads, The chromosomes separate in anaphase 1. The top chromosomes obtained has big F, and big G and bottom chromosome has big C, big D and big E (outlined in a rectangular box), and big B, big A. Anaphase 2 occurs and a caption reads, The sister chromatids separate in anaphase 2, forming four gametes. The gametes obtained below are: One normal non-recombinant gamete with the genes same as original. Two non-viable recombinant gametes where one has a copy of big G instead of big A gene. Another has big C, big D, and big E outlined in a rectangular box, while other genes are big A and big B. One non-recombinant gamete with pericentric inversion has big C, big D, and big E outlined in a rectangular box while other genes are big A, big B, big F, and big G. A caption below reads, Conclusion: Recombinant gametes are nonviable because genes are either missing or present in too many copies.

Figures 8.14 and 8.15 illustrate the results of single crossovers within inversions. Double crossovers in which both crossovers are on the same two strands (two-strand double crossovers) result in functional recombinant chromosomes. (To see why functional gametes are produced by double crossovers, try drawing the results of a two-strand double crossover.) Thus, even though the overall rate of recombination is reduced within an inversion, some viable recombinant progeny may still be produced through two-strand double crossovers. › **TRY PROBLEM 27**

IMPORTANCE OF INVERSIONS IN EVOLUTION

Inversions can play important evolutionary roles by suppressing recombination among a set of genes. As we have seen, crossing over within an inversion in an individual that is heterozygous for a pericentric or paracentric inversion leads to unbalanced gametes and no recombinant progeny. This suppression of recombination allows alleles adapted to a specific environment to remain together, unshuffled by recombination.

CONCEPTS

In an inversion, a segment of a chromosome is turned 180 degrees. Inversions cause breaks in some genes and may move others to new locations. In individuals heterozygous for a chromosome inversion, the homologous chromosomes form an inversion loop in prophase I of meiosis. When crossing over takes place within the inverted region, nonviable gametes are usually produced, resulting in a reduction in the recombination frequency observed.

✔ CONCEPT CHECK 3

A dicentric chromosome is produced when crossing over takes place in an individual heterozygous for which type of chromosome rearrangement?

a. Duplication
b. Deletion
c. Paracentric inversion
d. Pericentric inversion

Translocations

A **translocation** entails the movement of genetic material between nonhomologous chromosomes (see Figure 8.5d) or within the same chromosome. Translocation should not be confused with crossing over, in which there is an exchange of genetic material between *homologous* chromosomes.

In a **nonreciprocal translocation**, genetic material moves from one chromosome to another without any reciprocal exchange. Consider the following two nonhomologous chromosomes: AB•CDEFG and MN•OPQRS. If chromosome segment EF moves from the first chromosome to the second without any transfer of segments from the second chromosome to the first, a nonreciprocal translocation has taken place, producing chromosomes AB•CDG and MN•OPEFQRS. More commonly, there is a two-way exchange of segments between the chromosomes, resulting in a **reciprocal**

translocation. A reciprocal translocation between chromosomes AB•CDEFG and MN•OPQRS might give rise to chromosomes AB•CDQRS and MN•OPEFG.

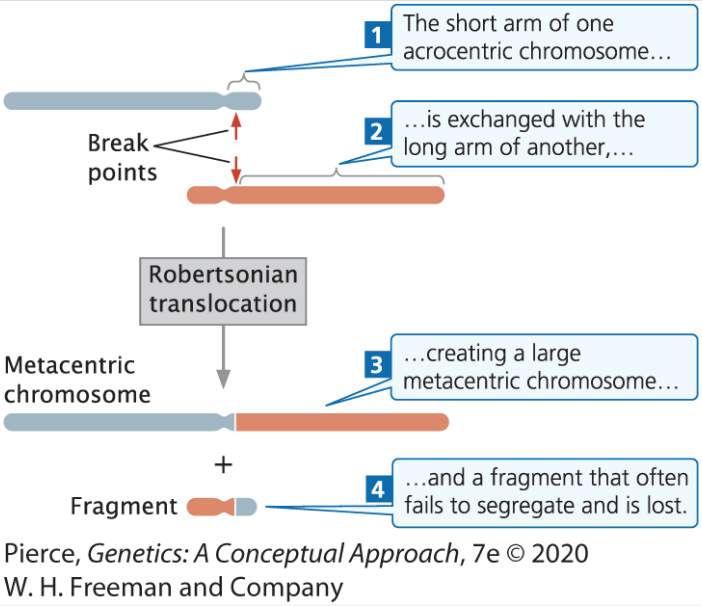
EFFECTS OF TRANSLOCATIONS

Translocations can affect a phenotype in several ways. First, they can physically link genes that were formerly located on different chromosomes. These new linkage relations may affect gene expression (a position effect), as genes translocated to new locations may come under the control of different regulatory sequences or other genes that affect their expression.

Second, the chromosome breaks that bring about translocations may take place within a gene and disrupt its function. Molecular geneticists have used these types of effects to map human genes. Neurofibromatosis, a genetic disease characterized by numerous fibrous tumors of the skin and nervous tissue, results from an autosomal dominant mutation. Linkage studies first placed the locus that, when mutated, causes neurofibromatosis on chromosome 17, but its precise location was unknown. Geneticists later narrowed down the location when they identified two patients with neurofibromatosis who possessed a translocation affecting chromosome 17. They assumed that these patients had developed neurofibromatosis because one of the chromosome breaks that occurred in the translocation disrupted a particular gene. Sequencing of DNA from the regions around the breaks eventually

led to the identification of the gene responsible for neurofibromatosis.

Deletions frequently accompany translocations. In a **Robertsonian translocation**, for example, the long arms of two acrocentric chromosomes become joined to a common centromere through a translocation, generating a metacentric chromosome with two long arms and another chromosome with two very short arms (**Figure 8.16**). The smaller chromosome is often lost because very small chromosomes do not have enough mass to segregate properly during mitosis and meiosis. The result is an overall reduction in chromosome number. As we will see, Robertsonian translocations are the cause of some cases of Down syndrome, a chromosome disorder discussed later in this chapter.

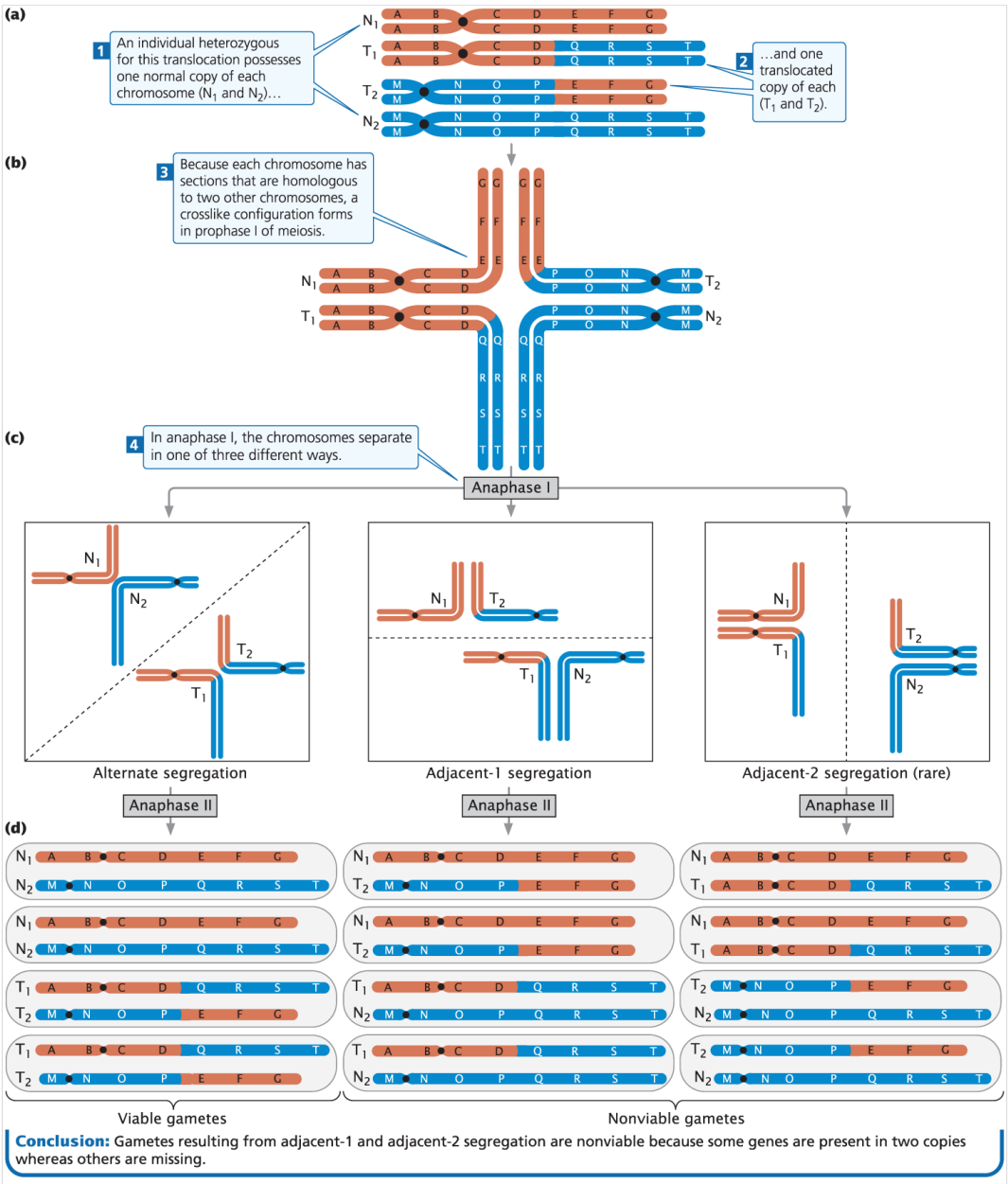


8.16 In a Robertsonian translocation, the short arm of one acrocentric chromosome is exchanged with the long arm of another.

Description
The diagram shows 2 acrocentric chromosomes arranged opposite to each other. The short arm of the top chromosome is above the long arm of bottom chromosome. Both these arms are labeled as break points. A caption reads, The short arm of one acrocentric chromosome is exchanged with the long arm of another creating a large metacentric chromosome and a fragment that often fails to segregate and is lost. Robertsonian translocation occurs and a large metacentric chromosome is obtained below. This large chromosome has larger arms on both sides of the centromere. The fragments join together as well.

TRANSLOCATIONS IN MEIOSIS

The effect of a translocation on chromosome segregation in meiosis depends on the nature of the translocation. Let's consider what happens in an individual heterozygous for a reciprocal translocation. Suppose that the original chromosomes are AB•CDEFG and M•NOPQRST (designated N_1 and N_2, respectively, for normal chromosomes 1 and 2) and that a reciprocal translocation takes place, producing chromosomes AB•CDQRST and M•NOPEFG (designated T_1 and T_2, respectively, for translocated chromosomes 1 and 2). An individual heterozygous for this translocation would possess one normal copy and one translocated copy of each chromosome (Figure 8.17a). Each of these chromosomes contains segments that are homologous to segments of two other chromosomes. Thus, when the homologous segments pair in prophase I of meiosis, crosslike configurations consisting of all four chromosomes form (Figure 8.17b).



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8.17 In an individual heterozygous for a reciprocal translocation, crosslike structures form in homologous pairing.

Description

Part a at the top shows 2 sets of 2 homologous chromosomes. For pair 1, the top chromosome named N subscript 1 is red and has genes big A, big B, big C, big D, big E, big F, and big G. Bottom chromosome named T subscript 1 is red has genes big A, big B, big C, big D, and a blue part with big Q, big R, big S, and big T. For pair 2, the top

chromosome named T subscript 2 is blue and has genes big M, big N, big O, big P and red part with big E, big F, and big G. The bottom chromosome named N subscript 2 has all blue part and has genes big M, big N, big O, big P, big Q, big R, big S, and big T. A caption reads, An individual heterozygous for this translocation possesses one normal copy of each chromosome, called N subscript 1 and N subscript 2, and one translocated copy of each, called T subscript 1 and T subscript 2.

Part b below shows the red chromosome N subscript 1 forming the shape of a bookend. One side of this chromosome is aligned with the red portion from the T subscript 1 chromosome, and the other side is aligned with red portion of the T subscript 2 chromosome. The blue chromosome is forming the same type of structure in inverse. This results in the shape of a cross. A caption reads, Because each chromosome has sections that are homologous to two other chromosomes, a cross-like configuration forms in prophase 1 of meiosis. Anaphase 1 occurs and leads to illustration c below. A caption reads, In anaphase 1, the chromosomes separate in one of three different ways.

Part c below shows three different ways of chromosome separation: 1, Alternate segregation where N subscript 1 and N subscript 2 are paired opposite T subscript 1 and T subscript 2. 2, Adjacent-1 segregation where N subscript 1 and T subscript 2 are together and T subscript 1 and N subscript 2 are together. 3, Adjacent-2 segregation (rare) where N subscript 1 and T subscript 1 are together and T subscript 2 and N subscript 2 are together.

Part d below shows viable gametes formed by alternate segregation and non-viable gametes formed by adjacent-1 and adjacent 2 segregations.

There are 4 viable gametes from alternate segregation as follows: N subscript 1, N subscript 2; N subscript 1, N subscript 2; T subscript 1, T subscript 2; and T subscript 1, T subscript 2.

4 non-viable gametes from adjacent-1 segregation are: N subscript 1, T subscript 2; N subscript 1, T subscript 2; T subscript 1, N subscript 2; and T subscript 1, N subscript 2.

4 non-viable gametes from adjacent-2 segregation are: N subscript 1, T subscript 1; N subscript 1, T subscript 1; T subscript 2, N subscript 2; and T subscript 2, N subscript 2.

A caption below reads, Conclusion: Gametes resulting from adjacent-I and adjacent-2 segregation are nonviable because some genes are present in two copies

whereas others are missing.

Notice that N_1 and T_1 have homologous centromeres (in both chromosomes, the centromere is between segments B and C); similarly, N_2 and T_2 have homologous centromeres (between segments M and N). Normally, homologous centromeres separate and move toward opposite poles in anaphase I of meiosis. With a reciprocal translocation, the chromosomes may segregate in three different ways. In **alternate segregation** (**Figure 8.17c**), N_1 and N_2 move toward one pole, and T_1 and T_2 move toward the opposite pole. In **adjacent-1 segregation**, N_1 and T_2 move toward one pole, and T_1 and N_2 move toward the other pole. In both alternate and adjacent-1 segregation, homologous centromeres segregate to opposite poles. **Adjacent-2 segregation**, in which N_1 and T_1 move toward one pole and T_2 and N_2 move toward the other, is rare because the two homologous chromosomes usually separate in meiosis.

The products of the three segregation patterns are illustrated in **Figure 8.17d**. As you can see, each of the gametes produced by alternate segregation possesses one complete set of the chromosome segments. These gametes are therefore functional and can produce viable progeny. In contrast, gametes produced by adjacent-1 and adjacent-2 segregation are not viable because some chromosome segments are present in two copies, whereas others are missing. Because adjacent-2 segregation is rare, most gametes are produced by alternate or adjacent-1 segregation. Therefore, approximately half of the gametes from an individual heterozygous

for a reciprocal translocation are expected to be functional. **› TRY PROBLEM 28**

THINK-PAIR-SHARE Question 2

CONCEPTS

In translocations, parts of chromosomes move to other nonhomologous chromosomes or to other regions of the same chromosome. Translocations can affect the phenotype by causing genes to move to new locations, where they come under the influence of new regulatory sequences, or by breaking genes and disrupting their function.

✔ CONCEPT CHECK 4

What is the outcome of a Robertsonian translocation?

a. Two acrocentric chromosomes
b. One large metacentric chromosome and one very small chromosome with two very short arms
c. One large metacentric chromosome and one large acrocentric chromosome
d. Two large metacentric chromosomes

Fragile Sites

Some chromosomes contain **fragile sites** (**Figure 8.18**), which are sites that develop constrictions or gaps when the cells are grown in culture and that are prone to breakage under certain conditions.

More than 100 fragile sites have been identified on human chromosomes.

Courtesy of
Dr. Christine Harrison.

8.18 Fragile sites are chromosomal regions susceptible to breakage under certain conditions. Shown here is a fragile site on the human X chromosome.

One of the most intensively studied rare fragile sites is located on the human X chromosome and is associated with **fragile-X syndrome**, a disorder that includes intellectual disability. Fragile-X syndrome, which exhibits X-linked inheritance and arises with a frequency of about 1 in 5000 male births, has been shown to result from an increase in the number of repeats of a CGG trinucleotide, a type of mutation known as expanding nucleotide repeats (see Chapter 18). In the case of fragile-X syndrome, the increased copies of the CGG trinucleotide occur within and disrupt a gene that encodes fragile X mental retardation protein (FMRP), which regulates the translation of other proteins and plays a role in the development of neural synapses.

Molecular studies of fragile sites have shown that many of these sites are more than 100,000 bp in length and include one or more genes. Fragile sites are often replicated late in S phase. At these sites, the enzymes that replicate DNA may stall while unwinding of the DNA continues (see Chapter 12), leading to long stretches of DNA that are unwound and vulnerable to breakage. In spite of recent advances in our understanding of fragile sites, their nature is not completely understood.

Copy-Number Variations

Chromosome rearrangements have traditionally been detected by examining chromosomes with a microscope. Visual examination identifies chromosome rearrangements on the basis of changes in the overall size of a chromosome, alteration of banding patterns revealed by chromosome staining, or the behavior of chromosomes in meiosis. Microscopy, however, can detect only large chromosome rearrangements, typically those that are at least 5 million base pairs in length.

With the completion of the Human Genome Project (see Chapter 20), detailed information about DNA sequences found on individual human chromosomes became available. Using this information, geneticists can now examine the number of copies of specific DNA sequences present in a cell and detect duplications, deletions, and other chromosome rearrangements that cannot be observed with microscopy alone. Because these methods measure the number of copies of particular DNA sequences, the variations that they detect are called **copy-number variations (CNVs)**. They include duplications and deletions that range in length from thousands of base pairs to several million base pairs. Many of these variants include at least one gene and may encompass several. Recently developed techniques for rapidly sequencing the genome now make it possible to detect not only copy-number variations but also other chromosome rearrangements, such as inversions and translocations. Chromosome rearrangements and copy-number variations are collectively referred to as **structural variants**.

Recent studies of copy-number variations have revealed that submicroscopic chromosome duplications and deletions are quite common: research suggests that each person may possess as many as a thousand copy-number variations. Many of these variations probably have no observable phenotypic effects, but some have now been implicated in a number of diseases and disorders. For example, Janine Wagenstaller and her colleagues studied copy-number variation in 67 children with unexplained intellectual disability and found that 11 (16%) of them had duplications or deletions. Copy-number variations have also been associated with osteoporosis, autism, schizophrenia, and a number of other diseases and disorders. › **TRY PROBLEM 20**

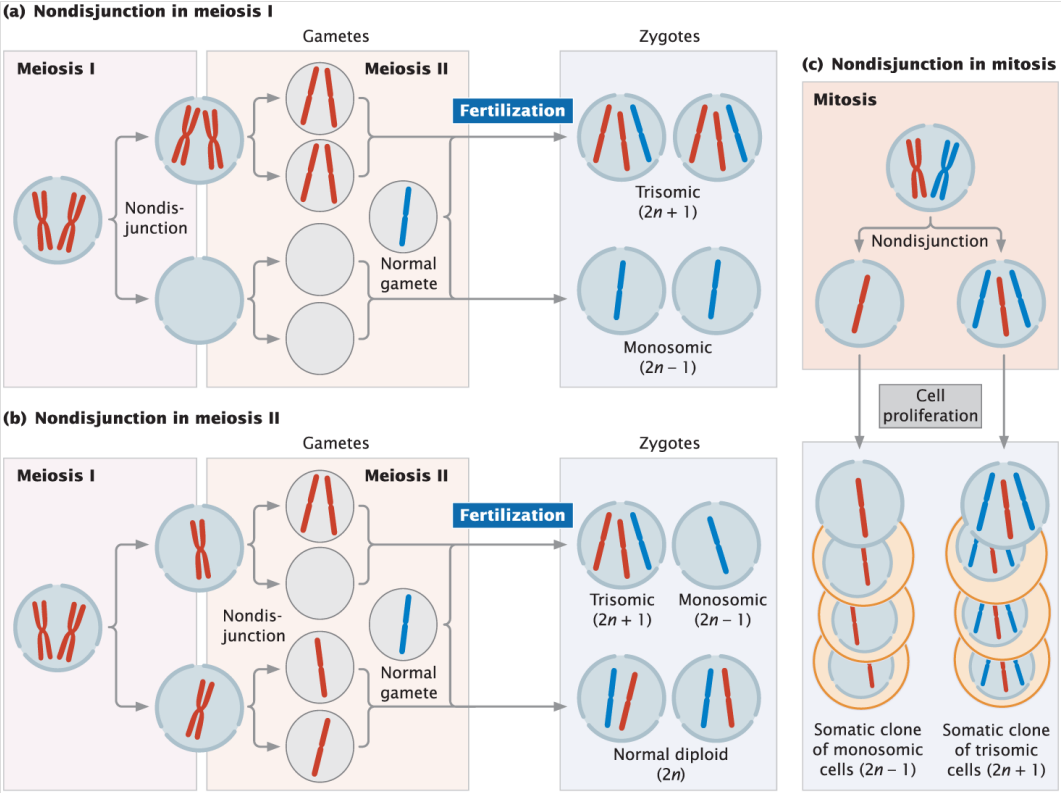
CONCEPTS

Variations in the number of copies of particular DNA sequences (copy-number variations) are surprisingly common in the human genome.

8.3 Aneuploidy Is an Increase or a Decrease in the Number of Individual Chromosomes

In addition to chromosome rearrangements, chromosome mutations include changes in the number of chromosomes. Variations in chromosome number can be classified into two basic types: **aneuploidy**, which is a change in the number of individual chromosomes, and **polyploidy**, which is an increase in the number of chromosome sets.

Aneuploidy can arise in several ways. First, a chromosome may be lost in the course of mitosis or meiosis if, for example, its centromere is deleted. Loss of the centromere prevents the spindle microtubules from attaching, so the chromosome fails to move to the spindle pole and does not become incorporated into a nucleus after cell division. Second, the small chromosome generated by a Robertsonian translocation may be lost in mitosis or meiosis. Third, aneuploidy may arise through nondisjunction, the failure of homologous chromosomes or sister chromatids to separate in meiosis or mitosis (see Chapter 4). Nondisjunction leads to some gametes or cells that contain an extra chromosome and other gametes or cells that are missing a chromosome (**Figure 8.19**).



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8.19 Aneuploids can be produced through nondisjunction in meiosis I, meiosis II, or mitosis. The gametes that result from meioses with nondisjunction may combine with a gamete (with blue chromosome) that results from normal meiosis to produce aneuploid zygotes.

Description

Part a at the top left shows non-disjunction during meiosis 1. In a box labeled meiosis 1, a nucleus has 2 X-shaped red chromosomes. Non-disjunction occurs and a nucleus with these 2 chromosomes and a nucleus with no chromosomes are produced. Meiosis 2 occurs and gametes are produced. Two gametes have 2 red chromatids each and 2 gametes contain no chromatids. A normal gamete has one blue chromatid. Normal gamete fertilizes with gametes with red chromatid and produces 2 trisomic (2 n plus 1) zygotes that have 2 red and one blue chromatid each. Normal gamete fertilizes with gametes with no chromatids and produces 2 monosomic zygotes (2 n minus 1) that have only one blue chromatid each.

Part b at the bottom left shows non-disjunction in meiosis 2. Here, normal separation of the 2 red X-shaped chromosomes occurs during meiosis 1. The gametes are as follows: one

gamete has 2 chromatids, one has no chromatid, while 2 gametes have 1 chromatid each. A normal gamete has blue chromatid. The normal gamete fertilizes with a gamete with two chromatids and other gamete with no chromatids. It produces 1 trisomic (2 n plus 1) zygote with 2 red and 1 blue chromatids and 1 monosomic zygote (2 n minus 1) with only 1 blue chromatid. The normal gamete fertilizes with gametes that have one chromatid each and produces 2 normal diploid (2 n) zygotes with one red and one blue chromatid each.

Part c at the top right shows non-disjunction in mitosis. A nucleus has 2 X-shaped chromosomes, one blue and one red. Non-disjunction occurs and a nucleus with 1 red chromatid and a nucleus with one red and 2 blue chromatids are produced. Cell proliferation occurs and a somatic clone of monosomic cells (2 n minus 1) and a somatic clone of trisomic cells (2 n plus 1) are produced.

Types of Aneuploidy

We consider four types of common aneuploid conditions in diploid organisms: nullisomy, monosomy, trisomy, and tetrasomy.

1. **Nullisomy** is the loss of both members of a homologous pair of chromosomes. It is represented as $2n - 2$, where n refers to the haploid number of chromosomes. Thus, among humans, who normally possess $2n = 46$ chromosomes, a nullisomic zygote has 44 chromosomes.
2. **Monosomy** is the loss of a single chromosome, represented as $2n - 1$. A human monosomic zygote has 45 chromosomes.
3. **Trisomy** is the gain of a single chromosome, represented as $2n + 1$. A human trisomic zygote has 47 chromosomes. The gain of a chromosome means that there are three homologous copies of

one chromosome. Most cases of Down syndrome, discussed later in this section, result from trisomy of chromosome 21.

4. **Tetrasomy** is the gain of two homologous chromosomes, represented as $2n + 2$. A human tetrasomic zygote has 48 chromosomes. Tetrasomy is not the gain of *any* two extra chromosomes but rather the gain of two homologous chromosomes, so that there are four homologous copies of a particular chromosome.

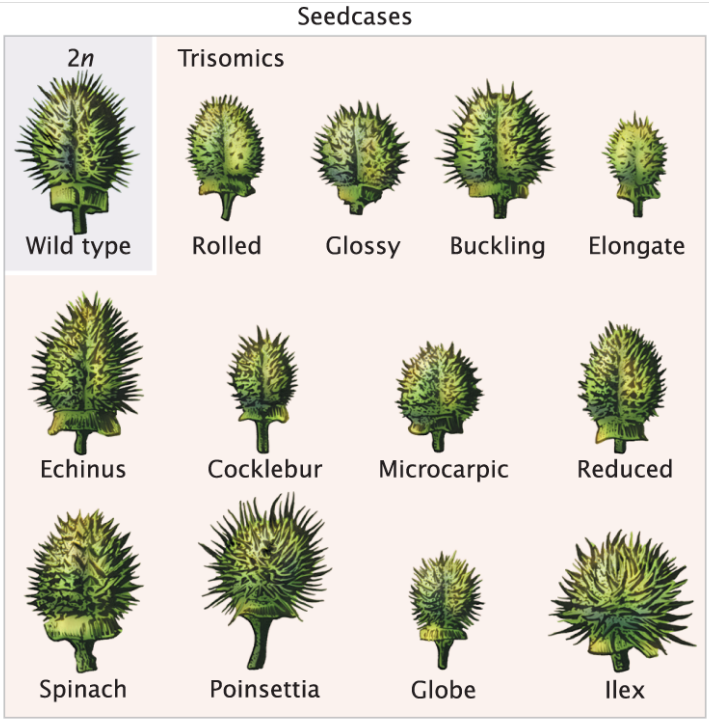
More than one aneuploid mutation may occur in the same individual. An individual that has an extra copy of each of two different (nonhomologous) chromosomes is referred to as being double trisomic; this condition is represented as $2n + 1 + 1$. Similarly, a double monosomic individual has two fewer nonhomologous chromosomes than normal ($2n - 1 - 1$), and a double tetrasomic individual has two extra pairs of homologous chromosomes ($2n + 2 + 2$).

THINK-PAIR-SHARE Question 3

Effects of Aneuploidy

One of the first aneuploids to be recognized was a fruit fly with a single X chromosome and no Y chromosome, discovered by Calvin Bridges in 1913 (see Chapter 4). Another early study of aneuploidy

focused on mutants in the Jimson weed (*Datura stramonium*). A. Francis Blakeslee began breeding this plant in 1913, and he observed that crosses with several Jimson-weed mutants produced unusual ratios of progeny. For example, the *globe* mutation (which produces a globe-shaped seedcase) was dominant but was inherited primarily from the female parent. When *globe* mutants self-fertilized, only 25% of the progeny had the globe phenotype. If the *globe* mutation were strictly dominant, Blakeslee should have seen 75% of the progeny with the trait (see Chapter 3), so the 25% that he observed was unusual. Blakeslee isolated 12 different mutants (**Figure 8.20**) that exhibited peculiar patterns of inheritance. Eventually, John Belling demonstrated that these 12 mutants were in fact trisomics. *D. stramonium* has 12 pairs of chromosomes ($2n = 24$), and each of the 12 mutants was trisomic for a different chromosome pair. The aneuploid nature of the mutants explained the unusual ratios that Blakeslee had observed in the progeny. Many of the extra chromosomes in the trisomics were lost in meiosis, so fewer than 50% of the gametes carried the extra chromosome, and the proportion of trisomics in the progeny was low. Furthermore, the pollen containing an extra chromosome was not as successful in fertilization, and trisomic zygotes were less viable.



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8.20 Mutant seedcases in Jimson weed (*Datura stramonium*) result from different trisomies. Each seedcase phenotype results from trisomy of a different chromosome.

Description

The wild type case seed is labeled as 2 n. The 12 different seed case mutants have different heights, widths, and levels of spikiness. These are labeled as trisomics and are as follows: Rolled, Glossy, Buckling, Elongate, Echinus, Cocklebur, Microcarpic, Reduced, Spinach, Poinsettia, Globe, and Ilex.

Aneuploidy usually alters the phenotype drastically. In most animals and many plants, aneuploidies are lethal. Because aneuploidy affects the number of gene copies but not their nucleotide sequences, the effects of aneuploidy are most likely due to abnormal gene dosage. Aneuploidy alters the dosage for some, but not all, genes, disrupting

the relative concentrations of gene products and often interfering with normal development.

A major exception to the relation between gene number and gene dosage pertains to genes on the mammalian X chromosome. In mammals, X-chromosome inactivation ensures that males (who have a single X chromosome) and females (who have two X chromosomes) receive the same functional dosage for X-linked genes (see Chapter 4 for further discussion of X-chromosome inactivation). Because additional X chromosomes in mammals are inactivated, we might expect that aneuploidy of the sex chromosomes would be less detrimental in these animals. Indeed, this is the case for mice and humans, for whom aneuploidy of the sex chromosomes is the most common form of aneuploidy seen in living organisms. Y-chromosome aneuploidy is probably relatively common because there is so little information in the Y chromosome.

THINK-PAIR-SHARE Question 4

CONCEPTS

Aneuploidy, the loss or gain of one or more individual chromosomes, may arise from the loss of a chromosome subsequent to translocation or from nondisjunction in meiosis or mitosis. It disrupts gene dosage and often has severe phenotypic effects.

✔ CONCEPT CHECK 5

A diploid organism has $2n = 36$ chromosomes. How many chromosomes will be found in a trisomic member of this species?

Aneuploidy in Humans

A high percentage of all human embryos that are conceived possess chromosome abnormalities. Findings from studies of women who are attempting pregnancy suggest that more than 30% of all conceptions are spontaneously aborted (miscarried), usually so early in development that the woman is not even aware of her pregnancy. Chromosome mutations are present in at least 50% of spontaneously aborted human fetuses, with aneuploidy accounting for most of them. This rate of chromosome abnormality in humans is higher than in other organisms that have been studied; in mice, for example, aneuploidy is found in no more than 2% of fertilized eggs. Most aneuploids in humans are due to missegregation of chromosomes in meiosis I during oogenesis, the formation of eggs in females.

In Chapter 2, we discussed the importance of crossovers for proper segregation of homologous chromosomes in meiosis I. Generally, each pair of homologous chromosomes must undergo at least one crossover for the two homologous chromosomes to segregate properly during meiosis. Crossovers connect the two chromosomes, creating tension when the homologs are pulled in opposite

directions during metaphase I (see Figure 2.19c). This tension is necessary for the two homologs to separate properly in anaphase I. Research of crossover formation in humans demonstrates that the process of crossover maturation in females is often inefficient compared to that in males, leading to fewer mature crossovers in females and, among those that do occur, more crossovers that are likely to lead to missegregation of chromosomes during meiosis. This may account for the high levels of aneuploidy seen in human conceptions that originate in oogenesis. Furthermore, researchers have identified a genetic variant at the *Polo-like Kinase 4* (*PLK4*) gene that increases the frequency of aneuploidy in humans. The *PLK4* gene plays a role in regulating the centriole (see Chapter 2), the disruption of which can lead to failure of the chromosomes to separate properly in mitosis. Interestingly, this genetic variant occurs at high frequencies in many human populations, and some evidence suggests it has been favored by natural selection, but why it might have been favored by natural selection is still unclear.

Aneuploidy in humans usually produces such serious developmental problems that spontaneous abortion results. Only about 2% of all fetuses with a chromosome mutation survive to birth.

THINK-PAIR-SHARE Question 5

SEX-CHROMOSOME ANEUPLOIDIES

The most common aneuploidies seen in living humans are those that involve the sex chromosomes. As is true of all mammals, aneuploidy of the human sex chromosomes is better tolerated than aneuploidy of autosomes. Both Turner syndrome and Klinefelter syndrome (see Figures 4.8 and 4.9) result from aneuploidy of the sex chromosomes.

AUTOSOMAL ANEUPLOIDIES

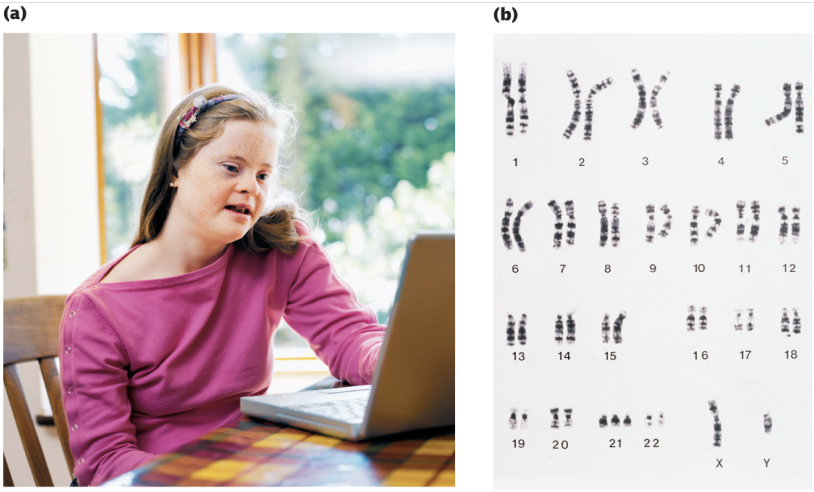
Autosomal aneuploidies resulting in live births are less common than sex-chromosome aneuploidies in humans, probably because there is no mechanism of dosage compensation for autosomes. Most embryos with autosomal aneuploidies are spontaneously aborted, though occasionally fetuses with aneuploidies of some of the small autosomes, such as chromosome 21, complete development. Because these chromosomes are small and carry relatively few genes, the presence of extra copies is less detrimental than it is for larger chromosomes.

DOWN SYNDROME

In 1866, John Langdon Down, physician and medical superintendent of the Earlswood Asylum in Surrey, England, noticed a remarkable resemblance among a number of his patients who were intellectually disabled: all of them possessed a broad, flat face, a small nose, and oval-shaped eyes. Their features were so similar, in

fact, that he felt that they might easily be mistaken for children from the same family. Down did not understand the cause of their intellectual disability, but his original description faithfully records the physical characteristics of people with this genetic form of intellectual disability. In his honor, the disorder is today known as Down syndrome.

Down syndrome, also known as **trisomy 21**, is the most common autosomal aneuploidy in humans (**Figure 8.21a**). The incidence of Down syndrome in the United States is similar to that worldwide—about 1 in 700 human births—although the incidence increases among children born to older mothers. Approximately 92% of those who have Down syndrome have three full copies of chromosome 21 (and therefore a total of 47 chromosomes), a condition termed **primary Down syndrome** (**Figure 8.21b**). Primary Down syndrome usually arises from spontaneous nondisjunction during egg formation: about 75% of the nondisjunction events that cause Down syndrome are maternal in origin, most arising in meiosis I. Most children with Down syndrome are born to unaffected parents, and the failure of the chromosomes to divide has little hereditary tendency. Therefore, a couple who has conceived one child with primary Down syndrome has only a slightly higher risk of conceiving a second child with Down syndrome (compared with other couples of similar age who have not had any children with Down syndrome). Similarly, the couple's relatives are not more likely to have a child with primary Down syndrome.



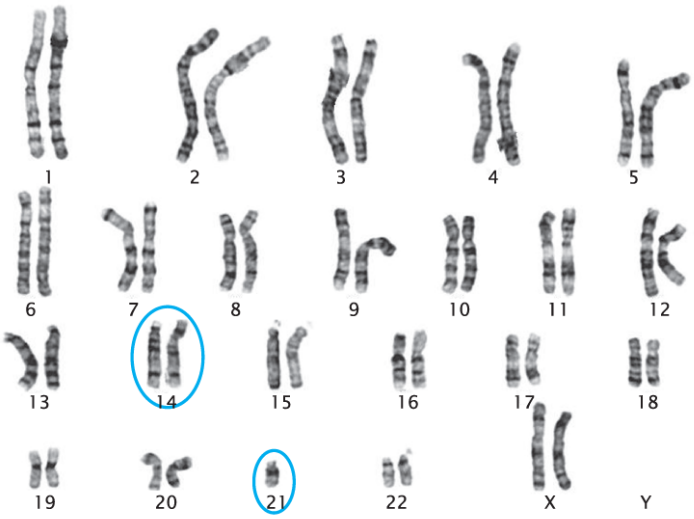
Part a: George Doyle/Getty Images. Part b: L. Wilatt, East Anglian Regional Genetics Service/ Science Photo Library/Science Source.

8.21 Down syndrome (a) is caused by trisomy of chromosome 21 (b).

About 4% of people with Down syndrome are not trisomic for a complete chromosome 21. Instead, they have 46 chromosomes, but an extra copy of part of chromosome 21 is attached to another chromosome through a translocation. This condition is termed **familial Down syndrome** because it has a tendency to run in families. The phenotypic characteristics of familial Down syndrome are the same as those of primary Down syndrome.

Familial Down syndrome arises in offspring whose parents are carriers of chromosomes that have undergone a Robertsonian translocation, most commonly between chromosome 21 and chromosome 14: the long arm of 21 and the short arm of 14 exchange places (Figure 8.22). This exchange produces one chromosome that includes the long arms of chromosomes 14 and 21 and another, very small chromosome that consists of the short arms of chromosomes 21 and 14. The small chromosome is generally lost after several cell

divisions. Although exchange between chromosomes 21 and 14 is the most common cause of familial Down syndrome, the condition can also be caused by translocations between 21 and other chromosomes, such as 15.



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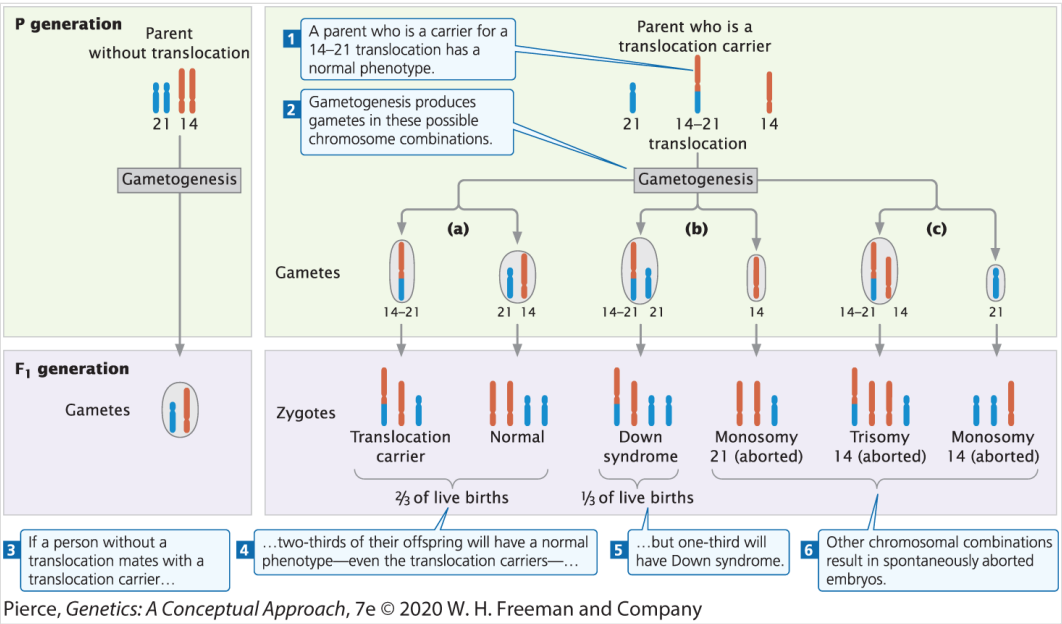
8.22 The translocation of part of chromosome 21 to another chromosome results in familial Down syndrome. Here, the long arm of chromosome 21 is attached to chromosome 14. This karyotype is from a translocation carrier, who is phenotypically normal but is at increased risk for producing children with Down syndrome.

People with this type of translocation, called **translocation carriers**, do not have Down syndrome. Although they possess only 45 chromosomes, their phenotypes are normal because they have two copies of the long arms of chromosomes 14 and 21, and apparently the short arms of these chromosomes (which are lost) carry no essential genetic information. Although translocation carriers have a

completely normal phenotype, they have an increased chance of producing children with Down syndrome.

When a translocation carrier produces gametes, the translocation chromosome segregates in one of three different ways. First, it may separate from the normal chromosomes 14 and 21 in anaphase I of meiosis (Figure 8.23a). In this type of segregation, half of the gametes produced will have the translocation chromosome and no other copies of chromosomes 21 and 14; the fusion of such a gamete with a normal gamete will give rise to a translocation carrier. The other half of the gametes produced by this first type of segregation will be normal, each with a single copy of chromosomes 21 and 14, and will result in offspring with a normal set of chromosomes.



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8.23 Translocation carriers are at increased risk for producing children with familial Down syndrome.

Description

The diagram shows P generation at the top with a parent without translocation who has normal chromosomes 21 and 14. Gametogenesis occurs and in F subscript 1 generation obtained below, a gamete produced has one 14 chromatid and one 21 chromatid. The illustration also shows a P generation at the top where parent is translocation carrier with one chromatid 21, one chromatid 14, and a hybrid chromatid 14-21. A caption reads, A parent who is a carrier for a 14 21 translocation has a normal phenotype. Gametogenesis occurs. A caption reads, Gametogenesis produces gametes in these possible chromosome combinations. Pair a obtained below has a gamete with 14-21 chromatid and a gamete with two chromatids 21 and 14. Pair b obtained below has a gamete with 14-21 chromatid and a 21 chromatid; another gamete has 14 chromatid. Pair c obtained below has a gamete with 14-21 chromatid and a 21 chromatid; another gamete has 21 chromatid.

Zygotes formed in the F subscript 1 generation below are as follows:

From Pair a:

A gamete with 14-21 chromatid produces a translocation carrier. A gamete with two chromatids 21 and 14 produces a normal zygote. These form two-third of a live birth.

From Pair b:

A gamete with 14-21 chromatid and a 21 chromatid produces a zygote with down syndrome. This forms one-third of live births. Another gamete has 14 chromatid and produces a zygote with monosomy 21 (aborted).

From Pair c:

A gamete with 14-21 chromatid and a 21 chromatid produces a zygote with trisomy 14 (aborted). Another gamete has 21 chromatid produces a zygote with monosomy 14 (aborted).

A caption reads, If a person without a translocation mates with a translocation carrier, two-thirds of their offspring will have a normal phenotype—even the translocation carriers, but one-third will have Down syndrome. Other chromosomal combinations result in spontaneously aborted embryos.

Alternatively, the translocation chromosome may separate from chromosome 14 and pass into the same cell with the normal chromosome 21 (**Figure 8.23b**). This type of segregation produces abnormal gametes only; half will have two functional copies of chromosome 21 (one normal and one attached to chromosome 14) and the other half will lack chromosome 21. If a gamete with the two functional copies of chromosome 21 fuses with a normal gamete carrying a single copy of chromosome 21, the resulting zygote will have familial Down syndrome. If a gamete lacking chromosome 21 fuses with a normal gamete, the resulting zygote will have monosomy 21 and will be spontaneously aborted.

In the third type of segregation, the translocation chromosome and the normal copy of chromosome 14 segregate together (**Figure 8.23c**). This pattern is presumably rare because the two centromeres are both derived from chromosome 14 and usually separate from each other. All the gametes produced by this process are abnormal: half result in monosomy 14 and the other half result in trisomy 14. All are spontaneously aborted.

Thus, only three of the six types of gametes that can be produced by a translocation carrier will result in the birth of a baby, and theoretically, these gametes should arise with equal frequency. One-third of the offspring of a translocation carrier should be translocation carriers like their parent, one-third should have familial Down syndrome, and one-third should have a normal set of chromosomes. In reality, however, fewer than one-third of the

children born to translocation carriers have Down syndrome, which suggests that some of the embryos with Down syndrome are spontaneously aborted. ❯ **TRY** **PROBLEM 33**

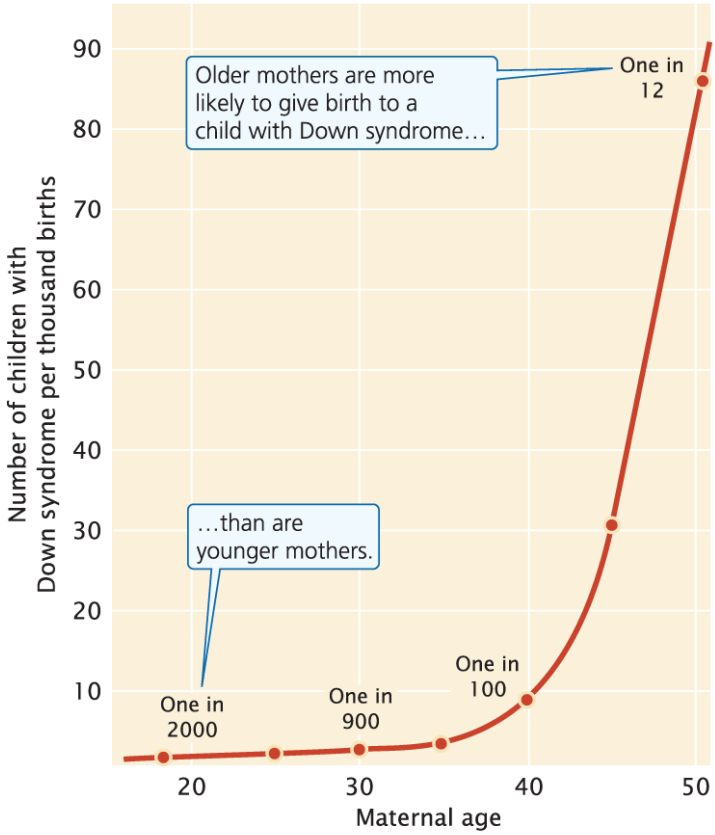
OTHER HUMAN TRISOMIES

Few autosomal aneuploidies in humans besides trisomy 21 result in live births. **Trisomy 18**, also known as **Edwards syndrome**, arises with a frequency of approximately 1 in 8000 live births. Babies with Edwards syndrome have severe intellectual disability, low-set ears, a short neck, deformed feet, clenched fingers, heart problems, and other disabilities. Few live for more than a year after birth. **Trisomy 13** has a frequency of about 1 in 15,000 live births and produces features that are collectively known as **Patau syndrome**. Characteristics of this condition include severe intellectual disability, a small head, a sloping forehead, small eyes, a cleft lip and palate, extra fingers and toes, and numerous other problems. About half of the children with trisomy 13 die within the first month of life, and 95% die by the age of three. Rarer still is **trisomy 8**, which arises with a frequency ranging from about 1 in 25,000 to 1 in 50,000 live births. This aneuploidy is characterized by intellectual disability, contracted fingers and toes, low-set malformed ears, and a prominent forehead. Most individuals born with this condition are mosaics, having some cells with three copies of chromosome 8 and other cells with the usual two copies (see the following discussion on genetic mosaicism). Complete trisomy 8, in which all cells in the

body have three copies of chromosome 8, usually results in spontaneous abortion.

ANEUPLOIDY AND MATERNAL AGE

Most cases of Down syndrome and other types of aneuploidy in humans arise from maternal nondisjunction, and the frequency of aneuploidy increases with maternal age (**Figure 8.24**). Why maternal age is associated with nondisjunction is not known for certain. Female mammals are born with primary oocytes suspended in the diplotene substage of prophase I of meiosis. Just before ovulation, meiosis resumes and the first division is completed, producing a secondary oocyte. At this point, meiosis is suspended again and remains so until the secondary oocyte is penetrated by a sperm. The second meiotic division takes place immediately before the nuclei of egg and sperm unite to form a zygote.



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8.24 The incidence of primary Down syndrome and other aneuploidies increases with maternal age.

Description

The horizontal axis represents maternal age from 20 to 50 with an interval of 10. The vertical axis represents number of children with Down syndrome per 1000 births and ranges from 0 to 90 with intervals of 10. The curve in the graph remains near the horizontal axis and starts to slope up at around age 35 and from age 40 to 50, there is a steep rise in the curve. The approximate data from graph are as follows:

For 20 years, 1 child in 2000 suffers from Down syndrome; for 30 years, 1 child in 900 suffers from Down syndrome; for 40 years, 1 child in 100 suffers from Down syndrome; and for 50 years, 1 child in 12 suffers from Down syndrome. A caption in the graph reads: Older mothers are more likely to give birth to a child with Down syndrome than are younger mothers.

Thus, primary oocytes may remain suspended in diplotene for many years before ovulation takes place and meiosis recommences. Cohesin (see Chapter 2) components of the spindle and other structures required for proper chromosome segregation may break down during the long arrest of meiosis, leading to more aneuploidy in children born to older mothers. No similar age effect is seen in males because sperm are produced continuously after puberty, with no long suspension of the meiotic divisions.

ANEUPLOIDY AND CANCER

Many tumor cells have extra chromosomes or missing chromosomes, or both; some types of tumors are consistently associated with specific chromosome mutations, including aneuploidy and chromosome rearrangements. The role of chromosome mutations in cancer will be explored in Chapter 23.

CONCEPTS

In humans, sex-chromosome aneuploidies are more common than autosomal aneuploidies. X-chromosome inactivation prevents problems of gene dosage for X-linked genes. Down syndrome results from three functional copies of chromosome 21, either through trisomy (primary Down syndrome) or a Robertsonian translocation (familial Down syndrome).

✔ CONCEPT CHECK 6

Briefly explain why, in humans and other mammals, sex-chromosome aneuploidies are more common than autosomal aneuploidies.

Uniparental Disomy

Normally, the two chromosomes of a homologous pair are inherited from two different parents—one from the father and one from the mother. The development of molecular techniques that facilitate the identification of specific DNA sequences (see Chapter 19) has made the determination of the parental origins of chromosomes possible. Surprisingly, these techniques have revealed that sometimes both chromosomes are inherited from the same parent, a condition termed **uniparental disomy**.

Many cases of uniparental disomy probably originate as trisomies. Although most autosomal trisomies are lethal, a trisomic embryo can survive if one of the three chromosomes is lost early in development. If, just by chance, the two remaining chromosomes are both from the same parent, uniparental disomy results.

Uniparental disomy violates the rule that children affected with a recessive disorder appear only in families in which both parents are carriers. For example, cystic fibrosis is an autosomal recessive disease; typically, both parents of an affected child are heterozygous for a cystic fibrosis mutation on chromosome 7. However, for a small proportion of people with cystic fibrosis, only one of the parents is heterozygous for a cystic fibrosis mutation. In these cases, cystic fibrosis is due to uniparental disomy: the person with cystic fibrosis has inherited two copies of the chromosome 7 that carries

the defective cystic fibrosis allele from the heterozygous parent and no copy of the normal allele from the other parent.

Uniparental disomy has also been observed in Prader–Willi syndrome, a rare condition that arises when a paternal copy of a gene on chromosome 15 is missing. Although most cases of Prader–Willi syndrome result from a chromosome deletion that removes the paternal copy of the gene (see Genomic Imprinting in Chapter 5), 20% to 30% of the cases arise when both copies of chromosome 15 are inherited from the mother and no copy is inherited from the father.

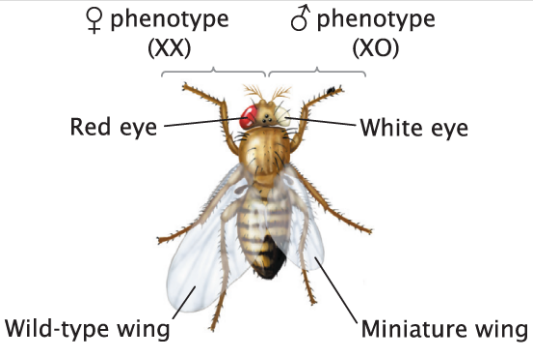
Genetic Mosaicism

Nondisjunction in a mitotic division may generate patches of cells in which every cell has a chromosome abnormality and other patches in which every cell has a normal karyotype. This type of nondisjunction leads to regions of tissue with different chromosome constitutions, a condition known as **genetic mosaicism** (see Chapter 6). Growing evidence suggests that genetic mosaicism is more common than is often recognized. For example, about 50% of those diagnosed with Turner syndrome (individuals with a single X chromosome) are actually mosaics, possessing some 45,X cells and some normal 46,XX cells. A few may even be mosaics for two or more abnormal karyotypes. The 45,X/46,XX mosaic usually arises

when an X chromosome is lost soon after fertilization in an XX embryo.

Fruit flies that are XX/XO mosaics (O designates the absence of a homologous chromosome; XO means that the cell has a single X chromosome and no Y chromosome) develop a mixture of male and female traits because the presence of two X chromosomes produces female traits, and the presence of a single X chromosome produces male traits (Figure 8.25). In fruit flies, sex is determined independently in each cell in the course of development. Those cells that are XX express female traits; those that are XO express male traits. Such sexual mosaics are called **gynandromorphs**. Normally, X-linked recessive genes are masked in heterozygous females, but in XX/XO mosaics, any X-linked recessive genes present in the cells with a single X chromosome will be expressed.

THINK-PAIR-SHARE Question 6



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8.25 Genetic mosaicism for the sex chromosomes produces a gynandromorph.
This XX/XO gynandromorph fruit fly carries one wild-type X chromosome and one

X chromosome with recessive alleles for white eyes and miniature wings. The left side of the fly has a normal female phenotype because the cells are XX and the recessive alleles on one X chromosome are masked by the presence of wild-type alleles on the other. The right side of the fly has a male phenotype with white eyes and miniature wings because the cells are missing the wild-type X chromosome (are XO), allowing the white and miniature alleles to be expressed.

Description

The gynandromorph fruit fly has female phenotype big X big X on the left side and male phenotype big X big O on the right side. On the left side, it has red eye and wild-type wing and on the right side, it has white eye and miniature wing.

CONCEPTS

In uniparental disomy, an individual organism has two copies of a chromosome from one parent and no copy from the other. Uniparental disomy may arise when a trisomic embryo loses one of the triplicate chromosomes early in development. In genetic mosaicism, different cells within the same individual organism have different chromosome constitutions.

8.4 Polyploidy Is the Presence of More Than Two Sets of Chromosomes

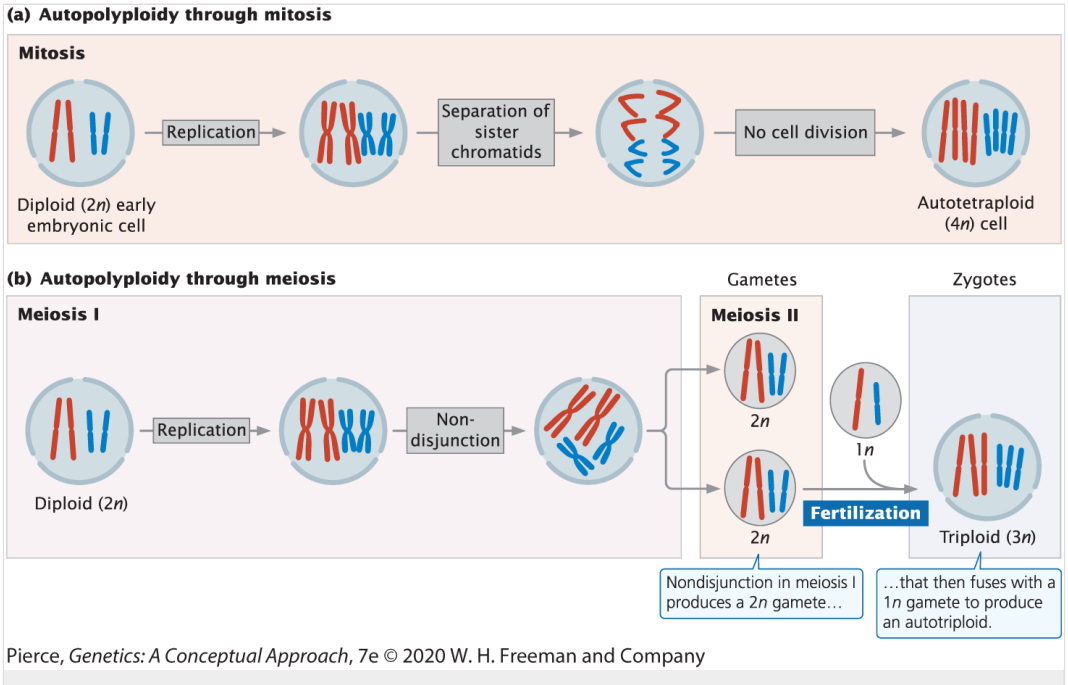
As discussed in the introduction to this chapter, some organisms (such as wheat) possess more than two sets of chromosomes—they are polyploid. Polyploids include *triploids* (3*n*), *tetraploids* (4*n*), *pentaploids* (5*n*), *hexaploids* (6*n*), and organisms with even higher numbers of chromosome sets.

Polyploidy is common in plants and is a major mechanism by which new plant species have evolved. Approximately 40% of all flowering-plant species and 70% to 80% of grasses are polyploids. A number of agriculturally important plants are polyploid, including wheat, oats, cotton, potatoes, and sugarcane. Polyploidy is less common in animals but is found in some invertebrates, fishes, salamanders, frogs, and lizards. No naturally occurring, viable polyploids are known in birds or mammals.

We consider two major types of polyploidy: **autopolyploidy**, in which all chromosome sets are from a single species, and **allopolyploidy**, in which chromosome sets are from two or more species.

Autopolyploidy

Autopolyploidy is caused by accidents of mitosis or meiosis that produce extra sets of chromosomes, all derived from a single species. Nondisjunction of all chromosomes in mitosis in an early 2*n* embryo, for example, doubles the chromosome number and produces an autotetraploid (4*n*), as depicted in Figure 8.26a. An autotriploid (3*n*) may arise when nondisjunction in meiosis produces a diploid gamete that then fuses with a normal haploid gamete to produce a triploid zygote (Figure 8.26b). Alternatively, triploids may arise from a cross between an autotetraploid that produces 2*n* gametes and a diploid that produces 1*n* gametes. Nondisjunction can be artificially induced by colchicine, a chemical that disrupts spindle formation. Colchicine is often used to induce polyploidy in agriculturally and ornamentally important plants.



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8.26 Autopolyploidy can arise through nondisjunction in mitosis or meiosis.

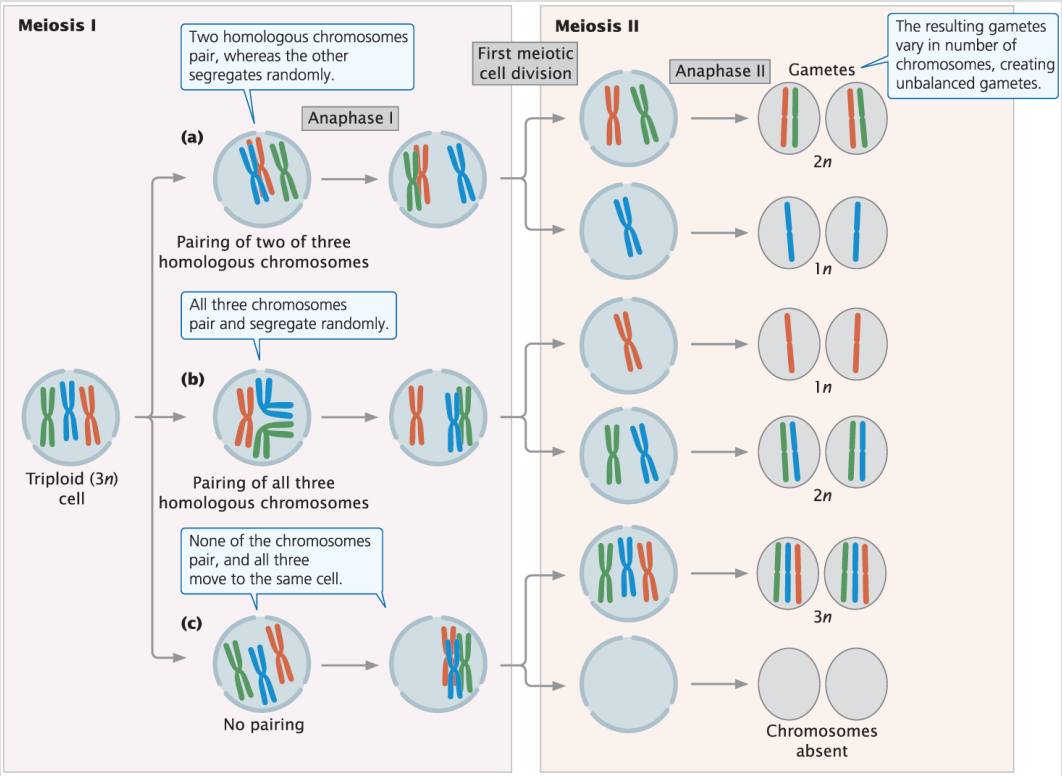
Description

Part a at the top shows autopolyploidy through mitosis. The illustration shows a diploid (2 n) early embryonic cell with 2 longer red and 2 relatively short blue chromatids. Replication occurs and the cell has 2 red large chromosomes and 2 blue smaller chromosomes. Separation of sister chromatids occurs and 4 chromatids move towards the right and 4 move towards the left of the cell. No cell division occurs and an autotetraploid (4 n) cell is produced. This cell has 4 longer red chromatids and 4 blue short chromatids.

Part b below shows a diploid (2 n) cell with 2 red longer and 2 blue short chromatids. Replication occurs and the cell has 2 red large chromosomes and 2 blue smaller chromosomes. Non-disjunction occurs and the chromosomes arrange randomly in the cell. Meiosis 2 occurs and gametes are produced. Each gamete is diploid (2 n) and has 2 red longer chromatids and 2 blue short chromatids each. A caption reads, Non-disjunction in meiosis I produces a 2 n gamete that then fuses with 1 n gamete to produce an autotriploid. One of these gametes is fertilized with a 1 n cell that has 1 red longer and 1 blue short chromatid. The zygote produced is a triploid (3 n) cell with 3 red longer and 3 blue short chromatids.

Because all the chromosome sets in autopolyploids are from the same species, they are homologous and attempt to align in prophase I of meiosis, which usually results in sterility. Consider meiosis in an autotriploid (**Figure 8.27**). In meiosis in a diploid cell, two homologs pair and align, but in autotriploids, three homologs are present. One of the three homologs may fail to align with the other two, and this unaligned chromosome will segregate randomly (see Figure 8.27a). Which gamete gets the extra chromosome will be determined by chance and will differ for each homologous trio of chromosomes. The resulting gametes will have two copies of some chromosomes and one copy of others. Even if all three chromosomes align, two

chromosomes must segregate to one gamete and one chromosome to the other (see Figure 8.27b). Occasionally, the presence of a third chromosome interferes with normal alignment, and all three chromosomes move to the same gamete (see Figure 8.27c).



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8.27 In meiosis in an autotriploid, homologous chromosomes can pair, or fail to pair, in three ways. This example illustrates the pairing and segregation of a single homologous set of chromosomes.

Description

In meiosis 1 at the left, the illustration shows a triploid cell (3 n) with three almost similar sized X-shaped chromosomes that are green, blue, and red. The illustration shows 3 different ways these chromosomes can segregate that are labeled as part a, b, and c.

Part a below shows pairing of two of three homologous chromosomes in meiosis 1 where the cell has red and blue pairing together while the green is aligned at a distance. A caption

reads, Two homologous chromosomes pair, whereas the other segregates randomly. Anaphase 1 occurs where a green and red pair together and blue remains at other end of the cell. First meiotic cell division occurs and next stage meiosis 2 is shown at the right. A cell has red and green chromosomes and another cell has only the blue chromosome. Anaphase 2 occurs and gametes are produced. The cell with the red and green chromosome produces 2 diploid (2 n) gametes with 1 red and 1 green chromatid each. The cell with blue chromosome produces 2 haploid (1 n) gametes with 1 blue chromatid each. A caption reads, The resulting gametes vary in number of chromosomes, creating unbalanced gametes.

Part b below shows pairing of all three homologous chromosomes in meiosis 1 where the cell has red, blue, and green chromosomes in close proximity with each other. This leads to cell where red chromosome is at one end while blue and green are paired together after anaphase 1. A caption reads, All three chromosomes pair and segregate randomly. First meiotic cell division occurs and next stage meiosis 2 is reached is shown at the right. One cell has only the red chromosome and another cell has blue and green chromosomes. Anaphase 2 occurs and gametes are produced. The cell with a red chromosome produces 2 haploid (1 n) gametes with 1 red chromatid each. The cell with blue and green chromosomes produces 2 diploid (2 n) gametes with 1 blue and 1 green chromatid each.

Part c below shows no pairing in meiosis 1where the cell has red, blue, and green chromosomes at a distance from each other. This leads to a cell where all the chromosomes move to the same side in the cell during anaphase 1. A caption reads, None of the chromosomes pair and all three move to the same cell. First meiotic cell division occurs and next stage meiosis 2 is reached is shown at the right. A cell has all three chromosomes and a cell has no chromosome. Anaphase 2 occurs and gametes are produced. The cell with all three chromosomes produces 2 (3 n) gametes with 1 blue, 1 red, and 1 green chromatid each. The cell with no chromosome produces 2 gametes with no chromosomes.

No matter how the three homologous chromosomes align, their random segregation will create **unbalanced gametes**, with various numbers of chromosomes. A gamete produced by meiosis in such an autotriploid might receive, say, two copies of chromosome 1, one

copy of chromosome 2, three copies of chromosome 3, and no copies of chromosome 4. When the unbalanced gamete fuses with a normal gamete (or with another unbalanced gamete), the resulting zygote has different numbers of the four types of chromosomes. This difference in number creates unbalanced gene dosage in the zygote, which is often lethal. For this reason, triploids do not usually produce viable offspring.

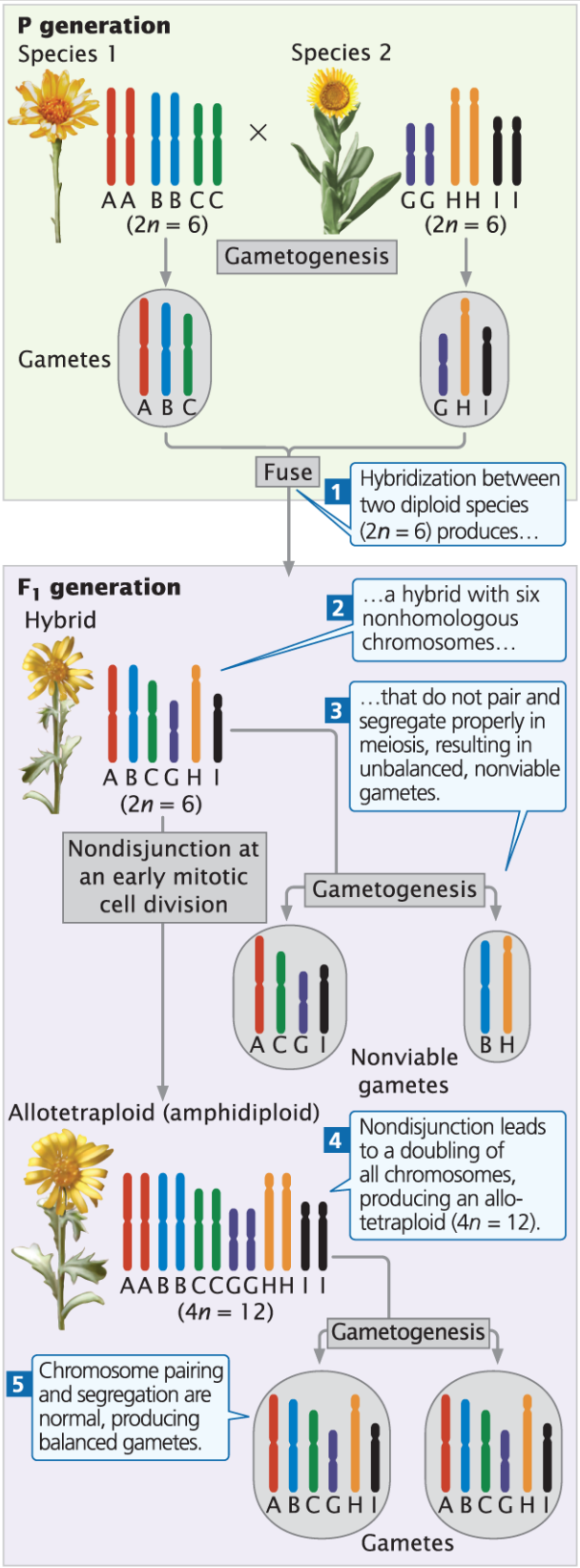
In even-numbered autopolyploids, such as autotetraploids, the homologous chromosomes can theoretically form pairs and divide equally. Equal division rarely takes place, however, so these types of autotetraploids also produce unbalanced gametes.

The sterility that usually accompanies autopolyploidy has been exploited in agriculture. Triploid bananas ($3n = 33$) are sterile and seedless. Similarly, seedless triploid watermelons have been created and are now widely sold.

Allopolyploidy

Allopolyploidy arises from hybridization between two species; the resulting polyploid carries chromosome sets derived from two or more species. **Figure 8.28** shows one mechanism for how allopolyploidy can arise from two species that are sufficiently related that hybridization takes place between them. Species I (AABBCC, $2n = 6$) produces haploid gametes with chromosomes ABC, and species

II (GGHHII, $2n = 6$) produces haploid gametes with chromosomes GHI. If gametes from species I and II fuse, a hybrid with six chromosomes (ABCGHI) is created. The hybrid has the same number of chromosomes as both diploid species, so the hybrid is considered diploid. However, because the hybrid chromosomes are not homologous, they will not usually pair and segregate properly in meiosis; thus, this hybrid is functionally haploid and sterile.



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8.28 Allopolyploids may arise from hybridization between two species followed by chromosome doubling.

Description
The diagram shows P generation at the top where species 1 is crossed with species 2. Species 1 has 3 pairs each of rod-shaped chromosomes big A, big B, and big C of varying lengths. A text reads, 2 n equals 6. Species 2 has 3 pairs each of rod-shaped chromosomes big G, big H, and big I of varying lengths. A text reads, 2 n equals 6. Gametogenesis occurs and the gametes are obatined below. The gametes produced by species 1 has one chromatid each of big A, big B, and big C. Gamete produced by species 2 has one chromatid each of big G, big H, and big I. These gametes fuse. A caption reads, Hybridization between two diploid species (2 n equals 6) produces a hybrid with six non-homologous chromosomes that do not pair and segregate properly in meiosis, resulting in unbalanced, non-viable gametes. A hybrid is obtained in F subscript 1 generation. The hybrid has one chromatid each of big A, big B, big C, big G, big H, and big I. A text reads, 2 n equals 6. This stage can progress in 2 conditions: gametogenesis and non-disjunction. First, gametogenesis occurs and 2 non-viable gametes are produced below. First gamete has one chromatid each of big A, big C, big G, and big I. Second gamete has one chromatid each of big B and big H. Second, non-disjunction at an early mitotic cell division occurs and an allotetraploid (amphidiploid) is produced below. This allotetraploid has a pair of each of chromosomes big A, big B, big C, big G, big H, and big I. A text reads, 4 n equals 12. A caption reads, Non-disjunction leads to a doubling of all chromosomes, producing an allotetraploid (4 n equals 12). Gametogenesis occurs and 2 gametes are produced. Both gametes have one chromatid each of big A, big B, big C, big G, big H, and big I. A caption reads, Chromosome pairing and segregation are normal, producing balanced gametes.

The sterile hybrid cannot produce viable gametes through meiosis, but it may be able to perpetuate itself through mitosis (asexual reproduction). On rare occasions, nondisjunction takes place in a mitotic division, which leads to a doubling of chromosome number and an allotetraploid with chromosomes AABBCCGGHHII. This type

of allopolyploid, consisting of two combined diploid genomes, is sometimes called an **amphidiploid**. Although the chromosome number has doubled compared with what was present in each of the parental species, the amphidiploid is functionally diploid: every chromosome has one and only one homologous partner, which is exactly what is required for proper segregation in meiosis. The amphidiploid can now undergo normal meiosis to produce balanced gametes with six chromosomes each. Allopolyploidy may also arise when nondisjunction during meiosis leads to a $2n$ gamete, which then fuses with a $1n$ or $2n$ gamete from a different species to produce a polyploid individual.

Georgii Karpechenko created polyploids experimentally in the 1920s. Cabbages (*Brassica oleracea*, $2n = 18$) and radishes (*Raphanus sativus*, $2n = 18$) are agriculturally important plants now, as they were then, but only the leaves of the cabbage and the roots of the radish are normally consumed. Karpechenko wanted to produce a plant that had cabbage leaves and radish roots so that no part of the plant would go to waste. Because both cabbages and radishes possess 18 chromosomes, Karpechenko was able to cross them successfully, producing a hybrid with $2n = 18$, but unfortunately the hybrid was sterile. After several crosses, Karpechenko noticed that one of his hybrid plants produced a few seeds. When planted, these seeds grew into plants that were viable and fertile. Analysis of their chromosomes revealed that the plants were allotetraploids, with $2n$

= 36 chromosomes. To Karpechenko's great disappointment, the new plants possessed the roots of a cabbage and the leaves of a radish.

WORKED PROBLEM

Species I has $2n = 14$ and species II has $2n = 20$. Give all possible chromosome numbers that may be found in the following individuals:

a. An autotriploid of species I.
b. An autotetraploid of species II.
c. An allotriploid formed from species I and species II.
d. An allotetraploid formed from species I and species II.

Solution Strategy

What information is required in your answer to the problem?

All possible chromosome numbers for individuals with the type of polyploidy indicated.

What information is provided to solve the problem?

- Species I has $2n = 14$ and species II has $2n = 20$.
- The type of polyploidy the individual possesses.

Solution Steps

The haploid number of chromosomes (n) for species I is 7, and that for species II is 10.

a. A triploid individual is $3n$. A common mistake is to assume that $3n$ means three times as many chromosomes as in a normal individual, but remember that normal individuals are $2n$. Because n for species I is 7 and all the chromosome sets of an autopolyploid are from the same species, $3n = 3 \times 7 = 21$.
b. An autotetraploid is $4n$ with all chromosome sets from the same species. The n for species II is 10, so $4n = 4 \times 10 = 40$.
c. A triploid is $3n$. By definition, an allopolyploid must have chromosome sets from two different species. An allotriploid could have $1n$ from species I and $2n$ from species II, or $(1 \times 7) + (2 \times 10) = 27$. Alternatively, it could have $2n$ from species I and $1n$ from species II, or $(2 \times 7) + (1 \times 10) = 24$. Thus, the number of chromosomes in an allotriploid could be 24 or 27.
d. A tetraploid is $4n$. By definition, an allotetraploid must have chromosome sets from at least two different species. An allotetraploid could have $3n$ from species I and $1n$ from species II, or $(3 \times 7) + (1 \times 10) = 31$; or $2n$ from species I and $2n$ from species II, or $(2 \times 7) + (2 \times 10) = 34$; or $1n$ from species I and $3n$ from species II, or $(1 \times 7) + (3 \times 10) = 37$. Thus, the number of chromosomes could be 31, 34, or 37.

» For additional practice, try **Problem 39** at the end of this chapter.

The Significance of Polyploidy

In many organisms, cell volume is correlated with nuclear volume, which in turn is determined by genome size. Thus, the increase in chromosome number in polyploidy is often associated with an increase in cell size, and many polyploids are physically larger than diploids. Breeders have used this effect to produce plants with larger leaves, flowers, fruits, and seeds. For example, as discussed in the introduction to the chapter, modern wheat is hexaploid ($6n = 42$) with chromosomes derived from three different wild species (Figure 8.1). As a result, the seeds of modern wheat are larger than those of its ancestors. Cultivated bananas are often polyploid. Most strains were created by crossing plants within and between two diploid species: *Musa acuminata* (genome = AA) and *Musa balbisiana* (genome = BB). Many cultivated strains are triploid, with three sets of chromosomes (AAA, AAB, or ABB), and others are tetraploid (AAAA, AAAB, AABB, or ABBB). Polyploid bananas generally have larger fruits than diploid bananas and many are seedless, making them more edible. Many other cultivated plants are also polyploid (**Table 8.2**).

TABLE 8.2 Examples of polyploid crop plants

Plant	Type of Polyploidy	Chromosome Sets	Chromosome Number
Potato	Autopolyploid	4*n*	48
Banana	Autopolyploid	3*n*	33
Peanut	Autopolyploid	4*n*	40
Sweet potato	Autopolyploid	6*n*	90

Tobacco	Allopolyploid	4*n*	48
Cotton	Allopolyploid	4*n*	52
Wheat	Allopolyploid	6*n*	42
Oats	Allopolyploid	6*n*	42
Sugarcane	Allopolyploid	8*n*	80
Strawberry	Allopolyploid	8*n*	56

Data from F. C. Elliot, *Plant Breeding and Cytogenetics* (New York: McGraw-Hill, 1958).

Polyploidy is less common in animals than in plants for several reasons. As discussed, allopolyploids require hybridization between different species, which happens less frequently in animals than in plants. Animal behavior often prevents interbreeding among species, and the complexity of animal development causes most interspecific hybrids to be nonviable. Many of the polyploid animals that do arise are in groups that reproduce through parthenogenesis (a type of reproduction in which the animal develops from an unfertilized egg). Thus, asexual reproduction may facilitate the development of polyploids, perhaps because the perpetuation of hybrid individuals through asexual reproduction provides greater opportunities for nondisjunction than does sexual reproduction. Only a few human polyploid babies have been reported, and most died within a few days of birth. Polyploidy—usually triploidy—is seen in about 10% of all spontaneously aborted human fetuses.

Although complete polyploidy, in which all cells are polyploid, is lethal in humans, significant numbers of autopolyploid cells do occur in some human tissues. For example, some liver cells and

heart cells are polyploid. Although the reason for polyploidy in these cells is unknown, the large cell size that results from polyploidy might be advantageous under certain circumstances. Polyploid cells are also found in many cancers. One study suggested that extra sets of chromosomes occur in 37% of all cancers.

THINK-PAIR-SHARE Question 7

IMPORTANCE OF POLYPLOIDY IN EVOLUTION

Polyploidy, particularly allopolyploidy, often gives rise to new species and has been particularly important in the evolution of flowering plants. Occasional genome doubling through polyploidy has been a major contributor to evolutionary success in several groups. For example, *Saccharomyces cerevisiae* (yeast) is a tetraploid, having undergone whole-genome duplication about 100 million years ago. The vertebrate genome has duplicated twice, once in the common ancestor of jawed vertebrates and again in the ancestor of fishes. Certain groups of vertebrates, such as some frogs and some fishes, have undergone additional duplications. Cereal plants have undergone several genome-duplication events.

The types of chromosome mutations we have discussed in this chapter are summarized in Table 8.3.

TABLE 8.3 Different types of chromosome mutations

Chromosome Mutation	Definition
Chromosome rearrangement	Change in chromosome structure
Chromosome duplication	Duplication of a chromosome segment
Chromosome deletion	Deletion of a chromosome segment
Inversion	Chromosome segment inverted 180 degrees
Paracentric inversion	Inversion that does not include the centromere in the inverted region
Pericentric inversion	Inversion that includes the centromere in the inverted region
Translocation	Movement of a chromosome segment to a nonhomologous chromosome or to another region of the same chromosome
Nonreciprocal translocation	Movement of a chromosome segment to a nonhomologous chromosome or to another region of the same chromosome without reciprocal exchange
Reciprocal translocation	Exchange between segments of nonhomologous chromosomes or between regions of the same chromosome
Aneuploidy	Change in number of individual chromosomes
Nullisomy	Loss of both members of a homologous pair
Monosomy	Loss of one member of a homologous pair
Trisomy	Gain of one chromosome, resulting in three homologous chromosomes
Tetrasomy	Gain of two homologous chromosomes, resulting in four homologous chromosomes
Polyploidy	Addition of entire chromosome sets
Autopolyploidy	Polyploidy in which extra chromosome sets are derived from the same species
Allopolyploidy	Polyploidy in which extra chromosome sets are derived from two or more species

CONCEPTS

Polyploidy is the presence of extra chromosome sets. Autopolyploids possess extra chromosome sets from the same species; allopolyploids possess extra chromosome sets from two or more species. Problems in chromosome pairing and segregation often lead to sterility in autopolyploids, but many allopolyploids are fertile.

✔ CONCEPT CHECK 7

Species A has $2n = 16$ and species B has $2n = 14$. How many chromosomes would be found in an allotriploid of these two species?

a. 21 or 24
b. 42 or 48
c. 22 or 23
d. 45

Chapter 8 Review

CHAPTER SUMMARY

Section 8.1 Learning Objective: Be able to define the major types of chromosome mutations.

- The three basic categories of chromosome mutations are: (1) chromosome rearrangements, which are changes in the structures of chromosomes; (2) aneuploidy, which is an increase or decrease in chromosome number; and (3) polyploidy, which is the presence of extra chromosome sets.

Section 8.2 Learning Objective: Be able to describe the major types of chromosome rearrangements and their effects.

- Chromosome rearrangements include duplications, deletions, inversions, and translocations.
- In individuals heterozygous for a duplication, the duplicated region forms a loop when homologous chromosomes pair in meiosis. Duplications often have pronounced effects on the phenotype owing to unbalanced gene dosage. Segmental duplications are common in the human genome.
- In individuals heterozygous for a deletion, one of the chromosomes loops out during pairing in meiosis. Deletions

may cause recessive alleles to be expressed.

- Pericentric inversions include the centromere; paracentric inversions do not. In individuals heterozygous for an inversion, the homologous chromosomes form inversion loops in meiosis, and reduced recombination is observed within the inverted region.
- In translocation heterozygotes, the chromosomes form crosslike structures in meiosis, and the segregation of chromosomes produces unbalanced gametes.
- Fragile sites are constrictions or gaps that appear at particular regions on the chromosomes of cells grown in culture and are prone to breakage under certain conditions.
- Copy-number variations (CNVs) are differences in the number of copies of DNA sequences and include duplications and deletions. These variants are common in the human genome; some are associated with diseases and disorders.

Section 8.3 Learning Objective: Be able to describe the different types of aneuploidy and their effects in humans.

- Nullisomy is the loss of two homologous chromosomes, monosomy is the loss of a single chromosome, trisomy is the addition of a single chromosome, and tetrasomy is the addition of two homologous chromosomes.
- Aneuploidy usually causes drastic phenotypic effects because it leads to unbalanced gene dosage.
- Primary Down syndrome is caused by the presence of three full copies of chromosome 21. Familial Down syndrome is caused

by the presence of two normal copies of chromosome 21 and a third copy that is attached to another chromosome through a translocation.
- Uniparental disomy is the presence of two copies of a chromosome from one parent and no copy from the other. Genetic mosaicism is caused by nondisjunction in an early mitotic division that leads to different chromosome constitutions in different cells of a single individual.

Section 8.4 Learning Objective: Be able to describe the different types of polyploidy and how they arise.

- All the chromosomes in an autopolyploid derive from one species; chromosomes in an allopolyploid come from two or more species.

WORKED PROBLEMS

Problem 1

A chromosome has the following segments, where • represents the centromere:

A B C D E • F G

What types of chromosome mutations are required to change this chromosome into each of the following chromosomes? (In some

cases, more than one chromosome mutation may be required.)

a. A B E • F G
b. A E D C B • F G
c. A B A B C D E • F G
d. A F • E D C B G
e. A B C D E E D C • F G

» Solution Strategy

What information is required in your answer to the problem?

The types of chromosome mutations that would lead to the chromosome shown.

What information is provided to solve the problem?

- The original segments found on the chromosome.
- The segments that are present after the mutations.

For help with this problem, review:

Section 8.2.

» Solution Steps

a. The mutated chromosome (A B E • F G) is missing segment CD, so this mutation is a deletion.

b. The mutated chromosome (A E D C B • F G) has one and only one copy of each original segment, but segment B C D E has been inverted 180 degrees. Because the centromere has not changed its location and is not in the inverted region, this chromosome mutation is a paracentric inversion.
c. The mutated chromosome (A B A B C D E • F G) is longer than normal, and we see that segment AB has been duplicated. This mutation is a tandem duplication.
d. The mutated chromosome (A F • E D C B G) is normal in length, but the order of the segments and the location of the centromere have changed; this mutation is therefore a pericentric inversion of region (B C D E • F).
e. The mutated chromosome (A B C D E E D C • F G) contains a duplication (C D E) that is also inverted, so this chromosome has undergone a duplication and a paracentric inversion.

Problem 2

Species I is diploid ($2n = 4$) with chromosomes AABB; related species II is diploid ($2n = 6$) with chromosomes MMNNOO. Give the chromosomes that would be found in individuals with the following chromosome mutations:

a. Autotriploidy in species I.
b. Allotetraploidy including species I and II.
c. Monosomy in species I.
d. Trisomy in species II for chromosome M.
e. Tetrasomy in species I for chromosome A.

f. Allotriploidy including species I and II.
g. Nullisomy in species II for chromosome N.

» Solution Strategy

What information is required in your answer to the problem?

The chromosomes found in individuals with each type of chromosome mutation.

What information is provided to solve the problem?

- Species I is diploid with $2n = 4$.
- Species I has chromosomes AABB.
- Species II is diploid with $2n = 6$.
- Species II has chromosomes MMNNOO.

For help with this problem, review:

Sections 8.3 and 8.4.

» Solution Steps

a. An autotriploid is $3n$, with all the chromosomes coming from a single species, so an autotriploid of species I would have chromosomes AAABBB ($3n = 6$).

Hint: First determine the haploid genome complement for each species. For species I, $n = 2$ with chromosomes AB, and for species II, $n = 3$ with chromosomes MNO.

b. An allotetraploid is $4n$, with the chromosomes coming from more than one species. An allotetraploid could consist of $2n$ from species I and $2n$ from species II, giving the allotetraploid ($4n = 2 + 2 + 3 + 3 = 10$) chromosomes AABBMMNNOO. An allotetraploid could also possess $3n$ from species I and $1n$ from species II ($4n = 2 + 2 + 2 + 3 = 9$; AAABBBMNO) or $1n$ from species I and $3n$ from species II ($4n = 2 + 3 + 3 + 3 = 11$; ABMMMNNNOOO).
c. A monosomic individual is missing a single chromosome, so monosomy in species I would result in $2n - 1 = 4 - 1 = 3$. The monosomy might include either of the two chromosome pairs, giving chromosomes ABB or AAB.
d. Trisomy requires an extra chromosome, so trisomy in species II for chromosome M would result in $2n + 1 = 6 + 1 = 7$ (MMMNNOO).
e. A tetrasomic individual has two extra homologous chromosomes, so tetrasomy in species I for chromosome A would result in $2n + 2 = 4 + 2 = 6$ (AAAABB).
f. An allotriploid is $3n$, with the chromosomes coming from two different species, so an allotriploid could be $3n = 2 + 2 + 3 = 7$ (AABBMNO) or $3n = 2 + 3 + 3 = 8$ (ABMMNNOO).
g. A nullisomic individual is missing both chromosomes of a homologous pair, so a nullisomy in species II for chromosome N would result in $2n - 2 = 6 - 2 = 4$ (MMOO).

IMPORTANT TERMS

chromosome mutation
metacentric
submetacentric
acrocentric
telocentric
chromosome rearrangement
chromosome duplication
tandem duplication
displaced duplication
reverse duplication
segmental duplication
unequal crossing over
chromosome deletion
pseudodominance
haploinsufficient
chromosome inversion
paracentric inversion
pericentric inversion
position effect
dicentric chromatid
acentric chromatid
dicentric bridge
translocation
nonreciprocal translocation

reciprocal translocation
Robertsonian translocation
alternate segregation
adjacent-1 segregation
adjacent-2 segregation
fragile site
fragile-X syndrome
copy-number variations (CNVs)
structural variants
aneuploidy
polyploidy
nullisomy
monosomy
trisomy
tetrasomy
Down syndrome (trisomy 21)
primary Down syndrome
familial Down syndrome
translocation carrier
Edwards syndrome (trisomy 18)
Patau syndrome (trisomy 13)
trisomy 8
uniparental disomy
genetic mosaicism
gynandromorph
autopolyploidy
allopolyploidy
unbalanced gametes

amphidiploid

ANSWERS TO CONCEPT CHECKS

1. a
2. Pseudodominance is the expression of a normally recessive mutation. It is produced when the dominant wild-type allele in a heterozygous individual is absent due to a deletion on one chromosome.
3. c
4. b
5. 37
6. Dosage compensation prevents the expression of additional copies of X-linked genes in mammals, and there is little information in the Y chromosome, so extra copies of the X and Y chromosomes do not have major effects on development. In contrast, there is no mechanism of dosage compensation for autosomes, and so extra copies of autosomal genes are expressed, upsetting developmental processes and causing the spontaneous abortion of aneuploid embryos.
7. c

Chapter 8 Assessment

COMPREHENSION QUESTIONS

Section 8.1

1. List the three basic categories of chromosome mutations, and define each one.

Section 8.2

2. Why do extra copies of genes sometimes cause drastic phenotypic effects?

3. Draw a pair of chromosomes as they would appear during synapsis in prophase I of meiosis in an individual heterozygous for a chromosome duplication.

4. What is haploinsufficiency?

5. What is the difference between a paracentric and a pericentric inversion?

6. How can inversions in which no genetic information is lost or gained cause phenotypic effects?

7. Explain, with the aid of a drawing, how a dicentric bridge is produced when crossing over takes place in an individual

heterozygous for a paracentric inversion.

8. Explain why recombination is suppressed in individuals heterozygous for paracentric and pericentric inversions.

9. How do translocations in which no genetic information is lost or gained produce phenotypic effects?

10. Sketch the chromosome pairing and the different segregation patterns that can arise in an individual heterozygous for a reciprocal translocation.

11. What is a Robertsonian translocation?

Section 8.3

12. List four major types of aneuploidy.

13. What is the difference between primary Down syndrome and familial Down syndrome? How does each type arise?

14. What is uniparental disomy, and how does it arise?

15. What is genetic mosaicism, and how does it arise?

Section 8.4

16. What is the difference between autopolyploidy and allopolyploidy? How does each arise?

17. Explain why autopolyploids are usually sterile, whereas allopolyploids are often fertile.

APPLICATION QUESTIONS AND PROBLEMS

Section 8.1

18. Examine the karyotypes shown in **Figure 8.2** and **Figure 8.3**. Are the individuals from whom these karyotypes were made males or females?

19. *Which types of chromosome mutations

    a. increase the amount of genetic material in a particular chromosome?

    b. increase the amount of genetic material in all chromosomes?

    c. decrease the amount of genetic material in a particular chromosome?

    d. change the position of DNA sequences in a single chromosome without changing the amount of genetic material?

    e. move DNA from one chromosome to a nonhomologous chromosome?

Section 8.2

20. *A chromosome has the following segments, where • represents the centromere:

    A B • C D E F G

What types of chromosome mutations are required to change this chromosome into each of the following chromosomes? (In some cases, more than one chromosome mutation may be required.)

a. A B A B • C D E F G

b. A B • C D E A B F G

c. A B • C F E D G

d. A • C D E F G

e. A B • C D E

f. A B • E D C F G

g. C • B A D E F G

h. A B • C F E D F E D G

i. A B • C D E F C D F E G

21. A chromosome initially has the following segments:

A B • C D E F G

Draw the chromosome, identifying its segments, that would result from each of the following mutations.

a. Tandem duplication of DEF

b. Displaced duplication of DEF

c. Deletion of FG

d. Paracentric inversion that includes DEFG

e. Pericentric inversion of BCDE

22. The following diagram represents two nonhomologous chromosomes:

A B • C D E F G

R S • T U V W X

What type of chromosome mutation would produce each of the following groups of chromosomes?

a. A B • C D

R S • T U V W X E F G

b. A U V B • C D E F G

R S • T W X

c. A B • T U V F G

R S • C D E W X

d. A B • C W G

R S • T U V D E F X

23. CONNECT *The *Notch* mutation is a deletion on the X chromosome of *Drosophila melanogaster*. Female flies heterozygous for *Notch* have an indentation on the margins of their wings; *Notch* is

lethal in the homozygous and hemizygous conditions. The *Notch* deletion covers the region of the X chromosome that contains the locus for white eyes, an X-linked recessive trait (see Chapter 4). Give the phenotypes and proportions of progeny produced in the following crosses.

a. A red-eyed Notch female is mated with a white-eyed male

b. A white-eyed Notch female is mated with a red-eyed male

c. A white-eyed Notch female is mated with a white-eyed male

24. The green-nose fly normally has six chromosomes: two metacentric and four acrocentric. A geneticist examines the chromosomes of an odd-looking green-nose fly and discovers that it has only five chromosomes; three of them are metacentric, and two are acrocentric. Explain how this change in chromosome number might have taken place.

25. *A wild-type chromosome has the following segments:

A B C • D E F G H I

Researchers have found individuals that are heterozygous for each of the following chromosome mutations. For each mutation, sketch how the wild-type and mutated chromosomes would pair in prophase I of meiosis, showing all chromosome strands.

a. A B C • D E F D E F G H I

b. A B C • D H I

c. A B C • D G F E H I

d. A B E D • C F G H I

26. For the chromosomes shown in **Figure 8.13**, draw the chromatids that would result from a two-strand double crossover: one crossover between C and D and the other crossover between D and E.

27.

*As discussed in this chapter, crossing over within a pericentric inversion produces chromosomes that have extra copies of some genes and no copies of other genes. The fertilization of gametes containing chromosomes with duplications or deletions often result in children with syndromes characterized by developmental delay, intellectual disability, abnormal development of organ systems, and early death. Maarit Jaarola and colleagues examined individual sperm cells of a male who was heterozygous for a pericentric inversion on chromosome 8 and determined that crossing over took place within the pericentric inversion in 26% of the meiotic divisions (M. Jaarola, R. H. Martin, and T. Ashley. 1998. *American Journal of Human Genetics* 63:218–224).

Assume that you are a genetic counselor (see Chapter 6) and that a couple seeks counseling from you. Both the man and the woman are phenotypically normal, but the woman is heterozygous for a pericentric inversion on chromosome 8. The man is karyotypically normal. What is the probability that

this couple will produce a child with a debilitating syndrome as the result of crossing over within the pericentric inversion?

28. *An individual heterozygous for a reciprocal translocation possesses the following chromosomes:

A B • C D E F G

A B • C D V W X

R S • T U E F G

R S • T U V W X

a. Draw the pairing arrangement of these chromosomes in prophase I of meiosis.

b. Diagram the alternate, adjacent-1, and adjacent-2 segregation patterns in anaphase I of meiosis.

c. Give the products that result from alternate, adjacent-1, and adjacent-2 segregation.

Section 8.3

29. CONNECT *Red–green color blindness is a human X-linked recessive disorder (see Chapter 4). A young man with a 47,XXY karyotype (Klinefelter syndrome) is color blind. His 46,XY brother is also color blind. Both parents have color vision. In which parent and where in meiosis did the nondisjunction that gave rise to the young man with Klinefelter syndrome take

place? Assume that no crossing over took place in prophase I of meiosis (see Chapter 2).

30.

Junctional epidermolysis bullosa (JEB) is a severe skin disorder that results in blisters over the entire body. The disorder is caused by autosomal recessive mutations at any one of three loci that help to encode laminin 5, a major component in the dermal–epidermal basement membrane. Leena Pulkkinen and colleagues described a male newborn who was born with JEB and died at 2 months of age (L. Pulkkinen et al. 1997. *American Journal of Human Genetics* 61:611–619); the child had healthy, unrelated parents. Chromosome analysis revealed that the infant had 46 normal-appearing chromosomes. Analysis of DNA showed that his mother was heterozygous for a JEB-causing allele at the *LAMB3* locus, which is on chromosome 1. The father had two normal alleles at this locus. DNA fingerprinting demonstrated that the male assumed to be the father had, in fact, conceived the child.

a. Assuming that no new mutations occurred in this family, explain the presence of an autosomal recessive disease in the child when the mother is heterozygous and the father is homozygous normal.

b. How might you go about proving your explanation? Assume that a number of genetic markers are available for each chromosome.

31.

In contrast to the fact that older women give birth to more children with Down syndrome, a few studies have demonstrated that children of *young* fathers are more likely to

have Down syndrome than those of older males. Other research has observed that the spermatocytes of juvenile mice have fewer crossovers (see Chapter 2) than the spermatocytes of adult male mice (M. J. Zelazowski et al. 2017. *Cell* 171:601–614). How might fewer crossovers in younger males result in higher rates of children with aneuploidy?

32.

Some people with Turner syndrome (see Chapter 4) are 45,X/46,XY mosaics. Explain how this mosaicism could arise.

33. *Bill and Betty have had two children with Down syndrome. Bill's brother has Down syndrome, and his sister has two children with Down syndrome. On the basis of these observations, indicate which of the following statements are most likely correct and which are most likely incorrect. Explain your reasoning.

a. Bill has 47 chromosomes.

b. Betty has 47 chromosomes.

c. Bill and Betty's children each have 47 chromosomes.

d. Bill's sister has 45 chromosomes.

e. Bill has 46 chromosomes.

f. Betty has 45 chromosomes.

g. Bill's brother has 45 chromosomes.

34. In mammals, sex-chromosome aneuploids are more common than autosomal aneuploids, but in fishes, sex-chromosome aneuploids and autosomal aneuploids are found with equal frequency. Offer a possible explanation for these differences between mammals and fishes. (Hint: Think about why sex-chromosome aneuploids are more common than autosomal aneuploids in mammals.)

35. *A young couple is planning to have children. Knowing that there have been a substantial number of stillbirths, miscarriages, and fertility problems on the husband's side of the family, they see a genetic counselor. A chromosome analysis reveals that whereas the woman has a normal karyotype, the man possesses only 45 chromosomes and is a carrier of a Robertsonian translocation between chromosomes 22 and 13.

    a. List all the different types of gametes that might be produced by the man.

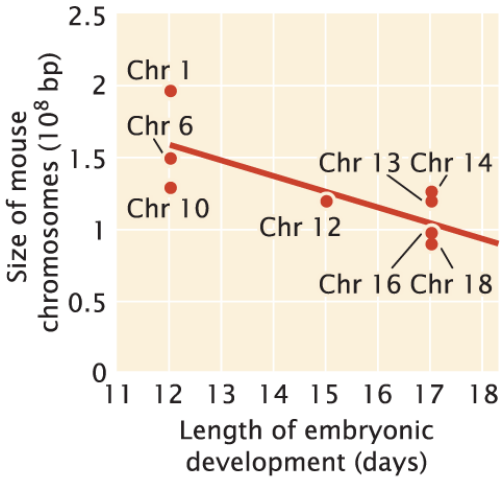
    b. What types of zygotes will develop when each of the gametes produced by the man fuses with a normal gamete produced by the woman?

    c. If trisomies and monosomies entailing chromosomes 13 and 22 are lethal, approximately what proportion of the surviving offspring are expected to be carriers of the translocation?

36.

Using breeding techniques, Andrei Dyban and V. S. Baranov (*Cytogenetics of Mammalian Embryonic Development*. Oxford: Oxford University Press, Clarendon Press; New York: Oxford University Press, 1987) created mice that were trisomic for each of the different mouse chromosomes. They found that only mice with

trisomy 19 developed. Mice that were trisomic for all other chromosomes died in the course of development. For some of these trisomics, the researchers plotted the length of development (number of days after conception before the embryo died) as a function of the size of the mouse chromosome that was present in three copies (see the adjoining graph). Summarize their findings, and provide a possible explanation for the results.



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[E. Torres, B. R. Williams, and A. Amon. 2008. *Genetics* 179:737–746, Fig. 2B.]

Description

The horizontal axis represents length of embryonic development in days from 11 to 18 with an interval of 1. The vertical axis represents size of mouse chromosomes in 10 to the power of 8 base pairs from 0 to 2 point 5 with an interval of 0 point 5. Dots on the graph represent chromosomes 1, 6, 10, 12, 13, 14, 16, and 18. The approximate data from the graph are as follows: Chromosome 1 corresponds to 12 days and size 2. Chromosome 6 corresponds to 12 days and size 1 point 5. Chromosome 10 corresponds to 12 days and size 1 point 3. Chromosome 12 corresponds to 15 days and size 1 point 2. Chromosome 13 and 14 corresponds to 17 days and size 1 point 2, and Chromosomes 16 and 18 corresponds to 17 days and size 1. A line starts from (12, 1 point 6) and ends at (18, 1).

Section 8.4

37. Species I has $2n = 16$ chromosomes. How many chromosomes will be found per cell in each of the following mutants in this species?

    a. Monosomic

    b. Autotriploid

    c. Autotetraploid

    d. Trisomic

    e. Double monosomic

    f. Nullisomic

    g. Autopentaploid

    h. Tetrasomic

38. Species I is diploid ($2n = 8$) with chromosomes AABBCCDD; related species II is diploid ($2n = 8$) with chromosomes MMNNOOPP. What types of chromosome mutations do individuals with the following sets of chromosomes have?

    a. AAABBCCDD

    b. MMNNOOOOPP

    c. AABBCDD

    d. AAABBBCCCDDD

e. AAABBCCDDD

f. AABBDD

g. AABBCCDDMMNNOOPP

h. AABBCCDDMNOP

39. *Species I has $2n = 8$ chromosomes and species II has $2n = 14$ chromosomes. What would the expected chromosome numbers be in individuals with the following chromosome mutations? Give all possible answers.

    a. Allotriploidy including species I and II

    b. Autotetraploidy in species II

    c. Trisomy in species I

    d. Monosomy in species II

    e. Tetrasomy in species I

    f. Allotetraploidy including species I and II

40. Suppose that species I in **Figure 8.28** had $2n = 10$ and species II in that figure had $2n = 12$. How many chromosomes would be present in the allotetraploid at the bottom of the figure?

41. Consider a diploid cell that has $2n = 4$ chromosomes: one pair of metacentric chromosomes and one pair of acrocentric chromosomes. Suppose that this cell undergoes nondisjunction, giving rise to an autotriploid cell ($3n$). The triploid cell then

undergoes meiosis. Draw the different types of gametes that could result from meiosis in the triploid cell, showing the chromosomes present in each type. To distinguish between the different metacentric and acrocentric chromosomes, use a different color to draw each metacentric chromosome; similarly, use a different color to draw each acrocentric chromosome. (Hint: See **Figure 8.27**.)

42. Assume that the autotriploid cell in **Figure 8.27** has $3n = 30$ chromosomes. For each of the gametes produced by this cell, give the chromosome number of the zygote that would result if the gamete fused with a normal haploid gamete.

43.

Nicotiana glutinosa ($2n = 24$) and *Nicotiana tabacum* ($2n = 48$) are two closely related plants that can be intercrossed, but the F_1 hybrid plants that result are usually sterile. In 1925, Roy Clausen and Thomas Goodspeed crossed *N. glutinosa* and *N. tabacum* and obtained one fertile F_1 plant (R. E. Clausen and T. H. Goodspeed. 1925. *Genetics* 10:278–284). They were able to self-pollinate the flowers of this plant to produce an F_2 generation. Surprisingly, the F_2 plants were fully fertile and produced viable seeds. When Clausen and Goodspeed examined the chromosomes of the F_2 plants, they observed 36 pairs of chromosomes in metaphase I and 36 individual chromosomes in metaphase II. Explain the origin of the F_2 plants obtained by Clausen and Goodspeed and the numbers of chromosomes observed.

44. What would be the chromosome number of progeny resulting from the following crosses in wheat (see **Figure 8.1**)? What type of

polyploid (allotriploid, allotetraploid, etc.) would result from each cross?

a. Einkorn wheat and Emmer wheat

b. Bread wheat and Emmer wheat

c. Einkorn wheat and bread wheat

45.

Karl and Hally Sax crossed *Aegilops cylindrica* ($2n = 28$), a wild grass found in the Mediterranean region, with *Triticum vulgare* ($2n = 42$), a type of wheat (K. Sax and H. J. Sax. 1924. *Genetics* 9:454–464). The resulting F_1 plants from this cross had 35 chromosomes. Examination of metaphase I in the F_1 plants revealed the presence of 7 pairs of chromosomes (bivalents) and 21 unpaired chromosomes (univalents).

a. If the unpaired chromosomes segregate randomly, what possible chromosome numbers will appear in the gametes of the F_1 plants?

b. What does the appearance of the bivalents in the F_1 hybrids suggest about the origin of *T. vulgare* wheat?

Left: Sam Brinker, MNR-NHIC, 2008/Canadian Food Inspection Agency.; right: Michael Hieber/ 123RF.com.

Aegilops cylindrica, jointed goatgrass. and *Triticum vulgare*, a type of wheat.

CHALLENGE QUESTIONS

Section 8.3

46. CONNECT Red–green color blindness is a human X-linked recessive disorder (see Chapter 4). Jill has normal color vision, but her father is color blind. Jill marries Tom, who also has normal color vision. Jill and Tom have a daughter who has Turner syndrome (Chapter 4) and is color blind.

    a. How did the daughter inherit color blindness?

    b. Did the daughter inherit her X chromosome from Jill or from Tom?

47. Progeny of triploid tomato plants often contain parts of an extra chromosome, in addition to the normal complement of 24 chromosomes (J. W. Lesley and M. M. Lesley. 1929. *Genetics* 14:321–336). Mutants with a part of an extra chromosome are referred to as secondaries. James and Margaret Lesley observed that secondaries arise from triploid ($3n$), trisomic ($3n + 1$), and double trisomic ($3n + 1 + 1$) parents, but never from diploids ($2n$). Give one or more possible reasons that secondaries arise from parents that have unpaired chromosomes but not from parents that are normal diploids.

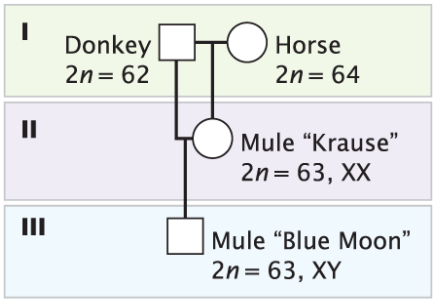
48. Mules result from a cross between a horse ($2n = 64$) and a donkey ($2n = 62$), have 63 chromosomes, and are almost always sterile. However, in the summer of 1985, a female mule named Krause, who was pastured with a male donkey, gave birth to a male foal (O. A. Ryder et al. 1985. *Journal of Heredity* 76:379–381). Blood tests established that the foal, appropriately named Blue Moon, was the offspring of Krause and that Krause was indeed a mule. Both Blue Moon and Krause were fathered by the same donkey (see the accompanying pedigree). The foal, like his mother, had 63 chromosomes—half of them horse chromosomes and the other half donkey chromosomes. Analyses of genetic markers showed that, remarkably, Blue Moon seemed to have inherited a complete set of horse chromosomes from his mother, instead of the random mixture of horse and donkey chromosomes that would be expected with normal meiosis. Thus, Blue Moon and Krause were not only mother and son but also brother and sister.

a. With the use of a diagram, show how, if Blue Moon inherited only horse chromosomes from his mother, Krause and Blue

Moon are mother and son as well as sister and brother.

b. Although rare, additional cases of fertile mules giving birth to offspring have been reported. In these cases, when a female mule mates with a male horse, the offspring is horselike in appearance, but when a female mule mates with a male donkey, the offspring is mulelike in appearance. Is this observation consistent with the idea that the offspring of fertile female mules inherit only a set of horse chromosomes from their mule mothers? Explain your reasoning.

c. Can you suggest a possible mechanism for how fertile female mules might pass on a complete set of horse chromosomes to their offspring?



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Description
Generation 1 shows a male donkey (2 n equals 62) crossed with a female horse (2 n equals 64). They have a female mule progeny named Krause in generation 2 (2 n equals 63, big X big X). The male donkey from generation 1 mates with Krause from generation 2 to have a male mule named Blue Moon in generation 3 (2 n equals 63, big X big Y).

Section 8.4

49. Humans and many other complex organisms are diploid, possessing two sets of genes, one inherited from the mother and one from the father. However, a number of eukaryotic organisms spend most of their life cycles in a haploid state. Many of these eukaryotes, such as *Neurospora* and yeast, still undergo meiosis and sexual reproduction, but most of the cells that make up the organism are haploid.

    Considering that haploid organisms are fully capable of sexual reproduction and generating genetic variation, why are most complex eukaryotes diploid? In other words, what might be the evolutionary advantage of existing in a diploid state instead of a haploid state? And why might a few organisms, such as *Neurospora* and yeast, exist as haploids?

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 8.1

1. Why do species usually have a characteristic number of chromosomes? Why don't we see many species in which chromosome number varies within the species, with some individuals having, say, $2n = 20$ and others having $2n = 24$?

Section 8.2

2. An individual is heterozygous for a reciprocal translocation, with the following chromosomes:
   A • B C D E F
   A • B C V W X
   R S T • U D E F
   R S T • U V W X

   a. Draw a picture of these chromosomes pairing in prophase I of meiosis.

   b. Draw the products of alternate, adjacent-1, and adjacent-2 segregations.

   c. Explain why the fertility of this individual is likely to be less than the fertility of an individual without a translocation.

Section 8.3

3. Monozygotic (identical) twins arise when a single egg, fertilized by a single sperm, divides early in development, giving rise to two genetically identical embryos (see Chapter 6). A recent study examined a pair of monozygotic twins in which one of the twins had trisomy 21 and the other twin had two normal copies of chromosome 21. Explain how two identical twins could differ in their number of chromosomes.

4. The human X chromosome is about the same size as human chromosomes 4 and 5. Trisomy of the X chromosome occurs about once in every thousand human female births, and yet trisomy 4 and trisomy 5 are almost never seen among living humans. Why do

these differences in the frequency of trisomic individuals occur, in spite of similar chromosome size?

5. Humans, compared to other organisms, exhibit a high level of chromosome nondisjunction resulting in aneuploid embryos, most of which are spontaneously aborted. Most of the nondisjunction occurs during oogenesis and is related to deficient crossover maturation in females. A specific genetic variant that increases the frequency of aneuploidy in humans has been discovered, and this variant occurs in high frequency in some human populations (see Aneuploidy in Humans in Section 8.3). These observations have led some researchers to speculate that the high frequency of chromosome nondisjunction observed in human females was adaptive in the past and was favored by natural selection. This might seem counterintuitive, because most aneuploids in humans are spontaneously aborted and result in lower fertility. Propose one or more explanations for why nondisjunction leading to aneuploidy might have been adaptive and favored by natural selection during human evolution.

6. In Chapter 2, we learned that the two chromosomes of a homologous pair carry information for the same characteristics but are not identical, having different alleles. Would you ever expect the two chromosomes of a homologous pair to be identical? If so, how might that occur? If not, why not?

Section 8.4

7. Trisomics often have more developmental problems than triploids. Can you suggest a reason why?

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Draw a concept map that illustrates the origins of modern wheat.

Section 8.2

2. Draw a concept map that compares and contrasts the results of chromosome pairing in individuals heterozygous for paracentric and pericentric inversions.

Section 8.3

3. Show the relationships among the different types of human aneuploidy by creating a concept map that includes at least the following terms: Klinefelter syndrome, sex-chromosome aneuploids, aneuploidy in humans, autosomal aneuploids, Down syndrome, Edwards syndrome, Turner syndrome, Patau syndrome, trisomy 8.

Sections 8.1, 8.2, and 8.3

4. Create a concept map that outlines the major types of chromosome mutations, including their differences and similarities. Include at least the following terms: autopolyploidy, chromosome mutation, monosomy, chromosome rearrangements, aneuploidy, duplication, deletion, inversion, allopolyploidy

translocation, paracentric inversion, pericentric inversion, reciprocal translocation, nonreciprocal translocation, trisomy, polyploidy, nullisomy, tetrasomy.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 9

Bacterial and Viral Genetic Systems

REUTERS/Rupak de Chowdhuri (INDIA).

Woman with leprosy, a disease caused by the bacterium *Mycobacterium leprae*. The study of *M. leprae* DNA isolated from ancient skeletons of medieval Europeans with leprosy has provided information about the evolution of this bacterium.

The Genetics of Medieval Leprosy

Leprosy, one of the most feared diseases in history, was well known in ancient times, and people with leprosy were frequently ostracized from society. Although leprosy is successfully treated today with antibiotics, it remains a major public health problem: from 2 million to 3 million people worldwide are disabled by leprosy, and over 200,000 new cases are reported each year. In its severest form, leprosy causes paralysis, blindness, and disfigurement. Leprosy is caused by the bacterium *Mycobacterium leprae*, which infects cells of the nervous system—although human genes do play a role in susceptibility to this disease.

In 2013, geneticists isolated DNA of *M. leprae* from five skeletons of medieval Europeans who exhibited signs of leprosy. Their comparisons of the gene sequences of these ancient bacteria with those of modern strains provided insight into the evolution of this organism. Scientists had previously determined the genome sequence of *M. leprae* and found that it contains 3,268,203 base pairs of DNA, 1 million base pairs fewer than the genomes of other mycobacteria. In most bacterial genomes, the vast majority of the DNA encodes proteins—little DNA lies between the protein-encoding genes. In contrast, only 50% of the DNA of *M. leprae* encodes proteins. *M. leprae* also has 2300 fewer genes than its close relative *M. tuberculosis*. An incredible 27% of *M. leprae*'s genome consists of nonfunctional copies of genes (called pseudogenes) that have been inactivated by mutations. Its reduced DNA content, fewer functional genes, and large number of pseudogenes suggest

that evolutionarily, the genome of *M. leprae* has undergone massive decay over time, losing DNA and acquiring mutations that have inactivated many of its genes. Although the reasons for this decay are not known, it helps account for *M. leprae*'s long generation time —14 days in humans, an incredibly long replication time for a bacterium—and the inability of scientists to culture the bacteria in the laboratory.

Leprosy was common in Europe until the Middle Ages, when it disappeared from the population. Why did it disappear from Europe, despite remaining common in many other parts of the world? To address this question, geneticists extracted DNA from the bones and teeth of five medieval skeletons (dating from the eleventh to the fourteenth centuries) exhumed from cemeteries in Denmark, Sweden, and the United Kingdom. They separated out the DNA of *M. leprae* and determined whole-genome sequences for the bacteria. They then compared the DNA sequences of these medieval strains of *M. leprae* with those of modern strains from India, Thailand, the United States, Brazil, and other locations.

This analysis revealed that the ancient strains of *M. leprae* were remarkably similar to modern strains. Three of the ancient strains were most closely related to modern strains from Iran and Turkey, suggesting a Middle Eastern–European connection to the disease. Some of the ancient strains were closely related to modern *M. leprae* currently found in the United States, suggesting that these North

American bacteria originated in Europe. The close similarity between ancient European strains and a modern virulent strain from North America suggests that leprosy did not disappear from Europe because it lost its virulence. More likely, improved social conditions, changes in the immunity of Europeans, or the presence of other infectious diseases brought about the demise of leprosy in Europe. This study of leprosy illustrates the importance of genetic studies of bacteria to human health and shows how modern tools of evolutionary and molecular genetics are being applied to our understanding of bacterial biology.

THINK-PAIR-SHARE

- In the ancient world, leprosy was greatly feared, and people with the disease were often ostracized from society. Why was leprosy so feared? Are there modern diseases that evoke similar fears and for which infected people are ostracized? If so, give some examples. What characteristics of a disease produce this response on the part of society? Are any of these modern diseases caused by bacteria or viruses?
- Genetic analysis of *M. leprae*, the bacterium that causes leprosy, reveals that its genome has undergone decay over time, losing DNA and acquiring mutations that make some of its genes nonfunctional. What might be some potential reasons for this evolutionary decay of its genome?

In this chapter, we examine some of the genetic properties of bacteria and viruses, and the mechanisms by which they exchange and recombine their genes.

9.1 Bacteria and Viruses Have Important Roles in Human Society and the World Ecosystem

The genetic systems of bacteria and viruses are studied because these organisms play critically important roles in human society and the world ecosystem. Viruses infect all living organisms and are, by far, the most common biological entity on Earth. They are the cause of many important diseases. Bacteria in the oceans produce 50% of the oxygen in the air and remove roughly 50% of the carbon dioxide. Bacteria and viruses also play critical roles in agriculture, serving as economically important pathogens of crops and domesticated animals, but also providing nitrogen, phosphorus, and other essential nutrients to our food plants. Bacteria are found naturally in the mouth, in the gut, and on the skin, where they are essential to human function and ecology. These complex communities of bacteria protect us from disease and are essential for digestion and other physiological functions.

Bacteria and viruses have immense medical significance because they cause many human diseases. Infectious diseases are one of the leading causes of death worldwide, although many of these diseases can be controlled through antibiotics and vaccines. Bacteria have also been harnessed to produce a number of economically important substances, including drugs, hormones, food additives,

and other chemicals and compounds. And viruses are now being used in gene therapies.

Bacteria and viruses are also important in the study of genetics because they possess a number of characteristics that make them suitable for genetic analysis (Table 9.1). Their simple genetic systems have a number of features in common with the genetic systems of humans and other more complex organisms, so information gleaned from the genetic study of bacteria and viruses often provides important insight into genetic principles that are applicable to many other organisms.

TABLE 9.1 Advantages of using bacteria and viruses for genetic studies

1. Reproduction is rapid.
2. Many progeny can be produced.
3. The haploid genome allows all mutations to be expressed directly.
4. Asexual reproduction simplifies the isolation of genetically pure strains.
5. Growth in the laboratory is easy and requires little space.
6. Genomes are small.
7. Techniques are available for isolating and manipulating their genes.
8. They have medical importance.
9. They can be genetically engineered to produce substances of commercial value.

Life in a Bacterial World

Humans like to think that we rule the world, but we are clearly in a minor position compared with bacteria. Bacteria first evolved some 3.5 billion years ago, 2 billion years before the first eukaryotes appeared (or perhaps even earlier, as suggested by some evidence). Today, bacteria are found in every conceivable environment, including boiling springs, highly saline lakes, and beneath more than 2 miles of ice in Antarctica. They are found at the top of Mount Everest and at the bottom of the deepest oceans. They are also present on and in *us*—in astounding numbers! Within the average human gut, there are approximately 10 trillion bacteria, ten times the total number of cells in the entire human body. No one knows how many bacteria populate the world, but an analysis conducted by scientists in 1998 estimated that the total number of living bacteria on Earth exceeded 5 million trillion trillion (5×10^{30}).

Bacteria are not only numerically vast but also constitute the majority of life's diversity. The total number of *described* species of bacteria is less than 10,000, compared with about 1.4 million plants, animals, fungi, and single-celled eukaryotes. But the number of described species of bacteria falls far short of the true microbial diversity.

Species of bacteria are typically described only after they have been cultivated and studied in the laboratory. Because only a few species are amenable to laboratory culture, it was impossible for many years to identify and study most bacteria. Then, in the 1970s,

molecular techniques for analyzing DNA became available and opened up a whole new vista on microbial diversity. These techniques revealed several important facts about bacteria. First, many of the relations among bacteria that microbiologists had worked out on the basis of their physical and biochemical traits turned out to be incorrect. Bacteria once thought to be related were in fact genetically quite different. Second, molecular analysis showed that members of one group of microbes—now called the archaea—are as different from other bacteria as they are from eukaryotes. Third, molecular analysis revealed that the number of different types of bacteria is astounding.

In 2007, Luiz Roesch and his colleagues set out to determine exactly how many types of bacteria exist in a gram of soil. They obtained soil samples from four locations: Brazil, Canada, and Florida and Illinois in the United States. From these soil samples, they extracted and purified bacterial DNA. Using this DNA, they determined the sequences of a gene present in all bacteria, the 16S rRNA gene. Each bacterial species has a unique 16S rRNA gene sequence, so the researchers could determine how many species of bacteria existed in each soil sample by counting the different DNA sequences.

Roesch's results were amazing. The number of different types of bacterial DNA in each gram of soil ranged from 26,140 for samples from Brazil to 53,533 for Canadian samples. Many unusual bacteria were detected, which appeared dissimilar to any previously described groups of bacteria. Another interesting finding was that

soil from agricultural fields harbored considerably fewer species than did soil from forests.

This study and others demonstrate that bacterial diversity far exceeds that of multicellular organisms, and undoubtedly, numerous groups of bacteria have yet to be discovered. In 2010, researchers founded the Earth Microbiome Project, the focus of which is to sample microbial communities in diverse environments across the globe, with the aim of increasing our understanding of the microbial world and how it is organized. Like it or not, we truly live in a bacterial world. ❯ **TRY PROBLEM 18**

THINK-PAIR-SHARE Question 1

Bacterial Diversity

Prokaryotes, as we saw in Chapter 2, are unicellular organisms that lack nuclear membranes and membrane-bounded organelles. For many years, biologists considered all prokaryotes to be related, but in recent years scientists have developed methods to isolate the DNA from bacteria that cannot be cultured in the laboratory and sequence their entire genome. This genome sequence information has revolutionized our understanding of microbial diversity. It is now clear that prokaryotes are divided into at least two distinct groups: the archaea and the eubacteria. The archaea are a group of

diverse prokaryotes that are found in almost all environments, including extreme environments such as hot springs and the bottom of oceans. The archaea are a very diverse group of microbes, consisting of at least four major groups and numerous phyla. The eubacteria (usually referred to simply as *bacteria*, as we generally do in this book) include most of the familiar bacterial species. Although superficially similar in their cell structure, bacteria and archaea are distinct in their genetic makeup, and the differences between them are as great as those between bacteria and eukaryotes. In fact, examination of their DNA sequences, as well as other evidence, indicates that archaea are more closely related to eukaryotes than to eubacteria. Some experts are now challenging the long-held idea that life consists of three major groups (eubacteria, archaea, and eukaryotes), suggesting instead that there are only two domains of life—eubacteria and archaea—and that eukaryotes are just a branch within the archaeal domain.

Bacteria are extremely diverse and come in a variety of shapes and sizes. Some are rod-shaped, whereas others are spherical or helical. Most are much smaller than eukaryotic cells, but at least one species isolated from the guts of fish is almost 1 mm long and can be seen with the naked eye. Some bacteria are photosynthetic. Others produce stalks and spores, superficially resembling fungi.

Bacteria have long been considered simple organisms that lack much of the cellular complexity of eukaryotes. However, evidence

now points to a number of similarities and parallels in bacterial and eukaryotic structure. For example, a bacterial protein termed FtsZ, which plays an integral part in bacterial cell division, is structurally similar to eukaryotic tubulin proteins, which are subunits of microtubules and help segregate chromosomes in mitosis and meiosis (see Chapter 2). Like eukaryotes, bacteria have proteins that help condense DNA. Other bacterial proteins function much as cytoskeletal proteins do in eukaryotes, helping give bacterial cells shape and structure. And although bacteria don't undergo mitosis and meiosis, replication of the bacterial chromosome precedes binary fission, and there are bacterial processes that ensure that one copy of the chromosome is allocated to each daughter cell.

CONCEPTS

Bacteria and viruses play important roles in human health and society and in the world ecosystem. They are extremely abundant and make up the majority of life's diversity. Analysis of DNA sequences allow study of bacteria that cannot be cultured in the laboratory.

9.2 Genetic Analysis of Bacteria Requires Special Methods

Heredity in bacteria is fundamentally similar to heredity in more complex organisms. However, the bacterial haploid genome and the small size of bacteria (which makes observation of their phenotypes difficult) necessitate different approaches and methods.

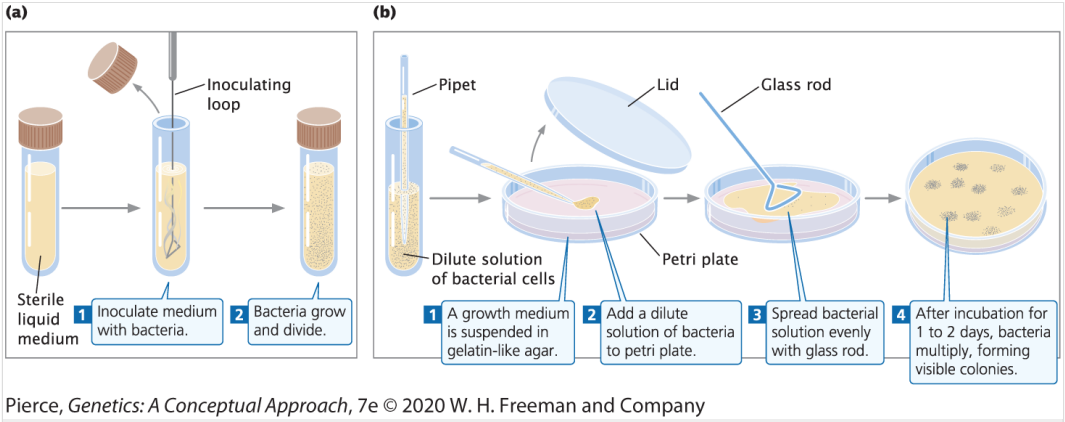
Techniques for the Study of Bacteria

Microbiologists have defined the nutritional needs of a number of bacteria and developed culture media for growing them in the laboratory. These culture media typically contain a carbon source, essential elements such as nitrogen and phosphorus, certain vitamins, and other required ions and nutrients. Wild-type, or **prototrophic**, bacteria can use these simple ingredients to synthesize all the compounds that they need for growth and reproduction. A medium that contains only the nutrients required by prototrophic bacteria is termed a **minimal medium**.

Mutant strains called **auxotrophs** lack one or more enzymes necessary for synthesizing essential compounds and will grow only on a medium supplemented with those essential molecules. For example, auxotrophic strains that are unable to synthesize the amino acid leucine will not grow on a minimal medium but *will*

grow on a medium to which leucine has been added. A **complete medium** contains all the substances, such as the amino acid leucine, required by bacteria for growth and reproduction.

Cultures of bacteria are often grown in test tubes that contain a sterile liquid medium, or *broth* (**Figure 9.1a**). A few bacteria are added to a broth tube, in which they grow and divide until all the nutrients are used up or—more commonly—until the concentration of their waste products becomes toxic to them. Bacteria can also be grown on agar plates (**Figure 9.1b**), in which melted agar is suspended in a growth medium and poured into the bottom half of a petri plate. The agar solidifies when cooled and provides a solid, gelatin-like base for bacterial growth. In a process called *plating*, a dilute solution of bacteria is spread over the surface of the agar. As each bacterium grows and divides, it gives rise to a visible clump of genetically identical cells (a **colony**). Genetically pure strains can be isolated by collecting bacteria from a single colony and transferring them to a new broth tube or agar plate. The chief advantage of this method is that it allows one to isolate and count bacteria, which individually are too small to see without a microscope.



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9.1 Growing bacteria in the laboratory. Bacteria can be grown (a) in a liquid medium (broth) or (b) on a solid medium.

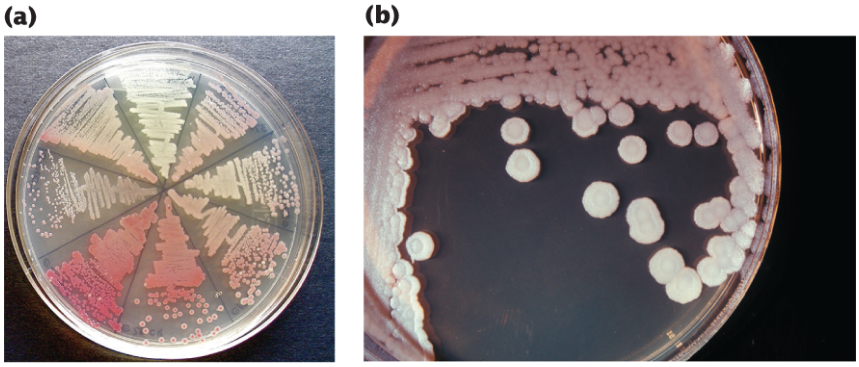
Description

Illustration a on the left shows a capped test tube with sterile liquid media inside. The cap of the test tube is removed and an inoculating loop is put inside the media and a caption reads, Inoculate medium with bacterium. The test tube is capped again and the liquid media shows dots inside and a caption reads, Bacteria grow and divide.

Illustration b on the right shows a test tube with bacteria in liquid media and a pipet is inserted into the test tube. The liquid media in the test tube is labeled dilute solution of bacterial cells. From the pipet, bacterial solution is inoculated on solid media in a petri plate. A caption pointing towards the petri plate reads, A growth medium is suspended in gelatin-like agar. Another caption pointing towards the bacterial suspension reads, Add a dilute solution of bacteria to petri plate. A lid is shown above the petri plate. Glass rod is used to stir the suspension onto the media. A caption reads Spread bacterial solution evenly with glass rod. Next petri plate shows several colonies of bacteria on the petri plate in circular pattern. A caption reads, After incubation for 1 to 2 days, bacteria multiply, forming visible colonies.

Microbiologists often study phenotypes that affect the appearance of the colony (Figure 9.2) or that can be detected by simple chemical tests. Auxotrophs are commonly studied phenotypes. Suppose we want to detect auxotrophs that cannot synthesize leucine (*leu*$^-$

mutants). We first spread bacteria on a petri plate containing a medium that includes leucine; both prototrophs that have the *leu*$^+$ allele and auxotrophs that have the *leu*$^-$ allele will grow on it (**Figure 9.3**). Next, using a technique called *replica plating*, we transfer a few cells from each of the colonies on the original plate to two new plates: one plate contains a medium to which leucine has been added; the other plate contains a medium lacking leucine. A medium that lacks an essential nutrient, such as the medium lacking leucine, is called a *selective* medium. The *leu*$^+$ bacteria will grow on both media, but the *leu*$^-$ mutants will grow only on the selective medium supplemented by leucine because they cannot synthesize their own leucine. Any colony that grows on a medium that contains leucine but not on a medium that lacks leucine consists of *leu*$^-$ bacteria. The auxotrophs that grow only on the supplemented medium can then be cultured for further study. Auxotrophic mutants are often used to study the results of genetic crosses and other genetic manipulations.

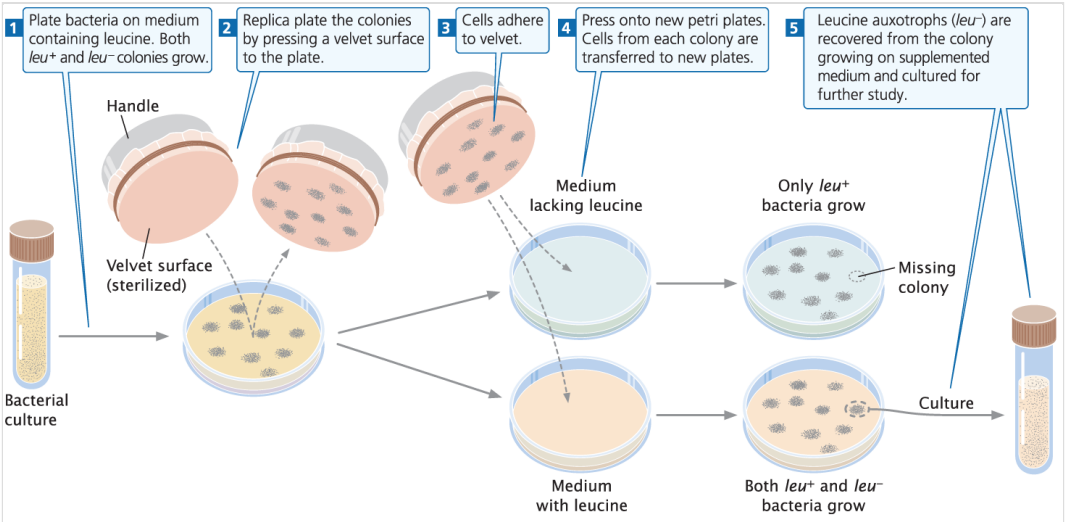


Part a: Courtesy Dr. Robert Shanks, Department of Ophthalmology, University of Pittsburgh. Part b: Biophoto Associates/Science Source.

9.2 Bacterial colonies have a variety of phenotypes. (a) *Serratia marcescens* with color variation. (b) *Bacillus cereus*; colony shape varies among different strains of this species.

Description

The photo a on the left shows bacterial growth in a petri plate with color variation ranging from white to dark orange. The photo b on the right shows round-shaped colonies of white color but different sizes.



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9.3 Mutant bacterial strains can be isolated on the basis of their nutritional requirements.

Description

The illustration shows bacteria grown on petri plates transferred to other plates via a velvet cloth that is tied to a circular handle almost the same size as the petri plate. The illustration starts with a bacterial culture in a capped test tube. The first caption reads, Plate bacteria on medium containing leucine. Both l e u superscript positive and l e u superscript negative colonies grow. Bacteria from the test tube are cultured on a petri plate and are transferred to a velvet cloth attached to a handle. The second caption reads, Replica plate the colonies by pressing a velvet surface to the plate. Third caption reads, Cells adhere to velvet. From the handle, bacteria are transferred to two petri-plates; one containing medium lacking leucine and one containing medium with leucine. The fourth caption reads, Press onto new petri plates. Cells from each colony are transferred to new plates. From these two petri-plates, two cultures are produced. The first has only l e u superscript positive bacterial colonies and the other has both l e u superscript positive and l e u superscript negative

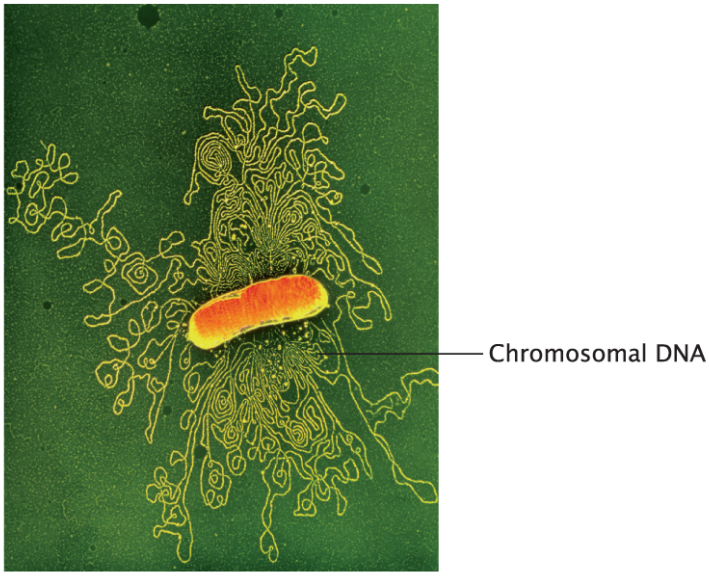
colonies. Missing colony is labeled in the first petri plate. From the second petri plate, the colony that was missing in first petri plate is cultured in a test tube. The fifth caption reads, Leucine auxotrophs, which are l e u superscript negative, are recovered from the colony growing on supplemented medium and cultured for further study. Text at the bottom reads, Conclusion: A colony that grows only on the supplemented medium has a mutation in a gene that encodes the synthesis of an essential nutrient.

Today, bacteria are often studied using genomic methods, which involve isolating and analyzing DNA sequences from bacteria (see Chapters 19 and 20). These methods make it possible to detect and study bacteria that cannot be cultured in the laboratory. They are providing insight into bacterial diversity, bacterial evolution, how genes are organized on the bacterial chromosome, and gene function.

The Bacterial Genome

Most bacterial genomes that have been studied consist of a circular chromosome that contains a single double-stranded DNA molecule, often several million base pairs (bp) in length (**Figure 9.4**). For example, the *Escherichia coli* genome has approximately 4.6 million base pairs of DNA. However, some bacteria contain multiple chromosomes. *Vibrio cholerae*, which causes cholera, has two circular chromosomes, and *Rhizobium meliloti* has three chromosomes. There are even a few bacteria that have linear chromosomes. Most bacterial chromosomes consist largely of sequences that encode proteins. For example, more than 90% of the

DNA in *E. coli* encodes proteins. In contrast, only about 1% of human DNA encodes proteins.



Dr. Gopal Murti/Science Source.

9.4 Most bacterial cells possess a single circular chromosome. The chromosome shown here is emerging from a ruptured bacterial cell.

Some newly discovered bacteria are smaller in size and have smaller genomes than bacteria that have been traditionally studied. The CPR (candidate phyla radiation) is a newly discovered group of bacteria that have been detected largely through culture-independent methods of DNA isolation and sequencing. The group is highly diverse and may contain as many as 90 different phyla. Species in CPR typically have a small genome, averaging about 1 million bp in length, and many are missing genes usually found in other bacteria. Based on their small cell size and reduced genomes, many bacteria in this group may be symbionts living on or in other bacteria or archaea.

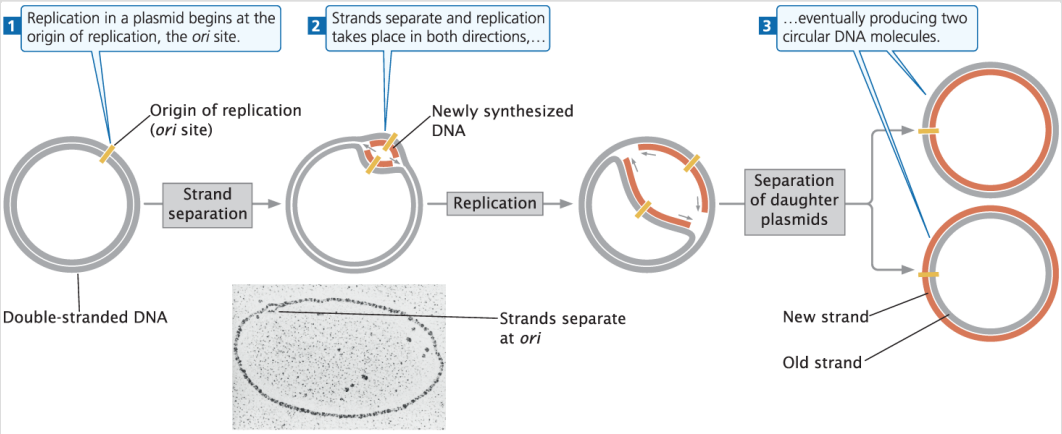
THINK-PAIR-SHARE Question 2

Plasmids

In addition to having a chromosome, many bacteria possess **plasmids**: small, usually circular DNA molecules that are distinct from the bacterial chromosome. Some plasmids are present in many copies per cell, whereas others are present in only one or two copies. In general, plasmids carry genes that are not essential to bacterial function but may play important roles in the life cycle and growth of their bacterial hosts. There are many different types of plasmids; *E. coli* alone is estimated to have more than 270 different types of naturally occurring plasmids. Some plasmids promote gene transfer between bacteria; others contain genes that kill other bacteria. Of importance to human health, plasmids are responsible for the spread of antibiotic resistance among bacteria. Plasmids are also used extensively in genetic engineering (see Chapter 19).

Most plasmids are circular and several thousand base pairs in length, although plasmids as large as several hundred thousand base pairs have also been found. Each plasmid possesses an origin of replication, a specific DNA sequence where DNA replication is initiated (see Chapter 2). The origin of replication allows a plasmid to replicate independently of the bacterial chromosome (**Figure 9.5**). **Episomes** are plasmids that are capable of replicating freely and are

able to integrate into bacterial chromosomes. The F (fertility) factor of *E. coli* (Figure 9.6) is an episome that controls mating and gene exchange between *E. coli* cells, a process we will discuss shortly.



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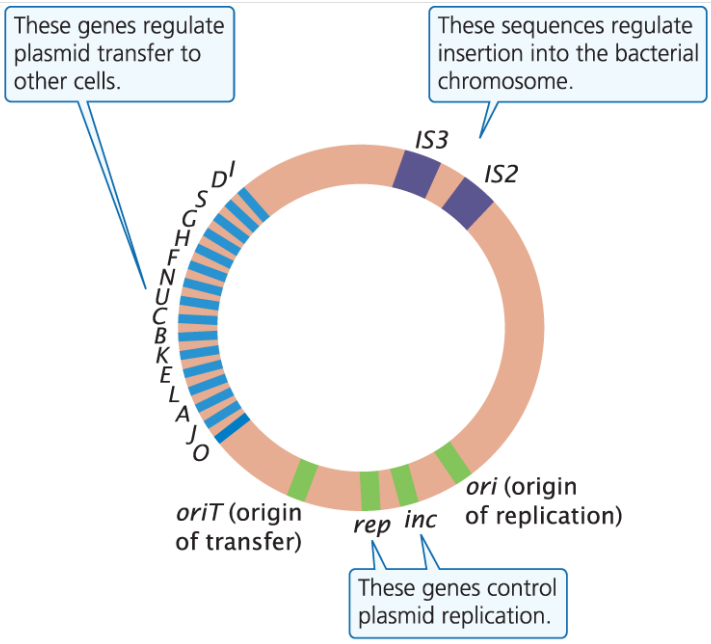
Photograph: Biology Pics/Science Source.

9.5 A plasmid replicates independently of its bacterial chromosome.
Replication begins at the origin of replication (*ori*) and continues around the circle. In this diagram, replication is taking place in both directions; in some plasmids, replication is in one direction only.

Description
The illustration shows a circular double-stranded D N A on which the origin of replication is marked with a yellow line. A caption pointing towards the ori reads, Replication in a plasmid begins at the origin of replication, the ori site. A forward arrow labeled strand separation points to the circular D N A with a bubble created at the ori. The second caption reads, Strands separate and replication takes place in both directions, eventually producing 2 circular D N A molecules. Another forward arrow labeled replication points to the D N A with its strands open up at the ori and newly synthesized D N A is shown next to the template D N A. Another forward arrow labeled separation of daughter plasmids points to two double-stranded circular daughter D N A molecules, each with a new strand and an old strand.



F factor

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9.6 The F factor, a circular episome of *E. coli*, contains a number of genes that regulate its transfer into a bacterial cell, replication, and insertion into the bacterial chromosome. Replication is initiated at *ori*. Insertion sequences (see Chapter 18) *IS3* and *IS2* control insertion into the bacterial chromosome and excision from it.

Description

A group of 16 genes named O, J, A, L, E, K, B, C, U, N, F, H, G, S, D, and I are labeled as genes that regulate plasmid transfer to other cells. The I S 3 and I S 2 genes are labeled as sequences that regulate insertion into the bacterial chromosome. The rep and inc genes are labeled as genes that control plasmid replication. There are also 2 ori's: one is an origin of replication and the other is an origin of transfer.

CONCEPTS

Bacteria can be studied in the laboratory by growing them on liquid or solid media. A typical bacterial genome consists of a single circular chromosome that contains several million base

pairs, although some bacteria have smaller genomes. Bacterial genes may be present on plasmids, which are small, circular DNA molecules that replicate independently of the bacterial chromosome.

✔ CONCEPT CHECK 1

Which of the following statements is true of plasmids?

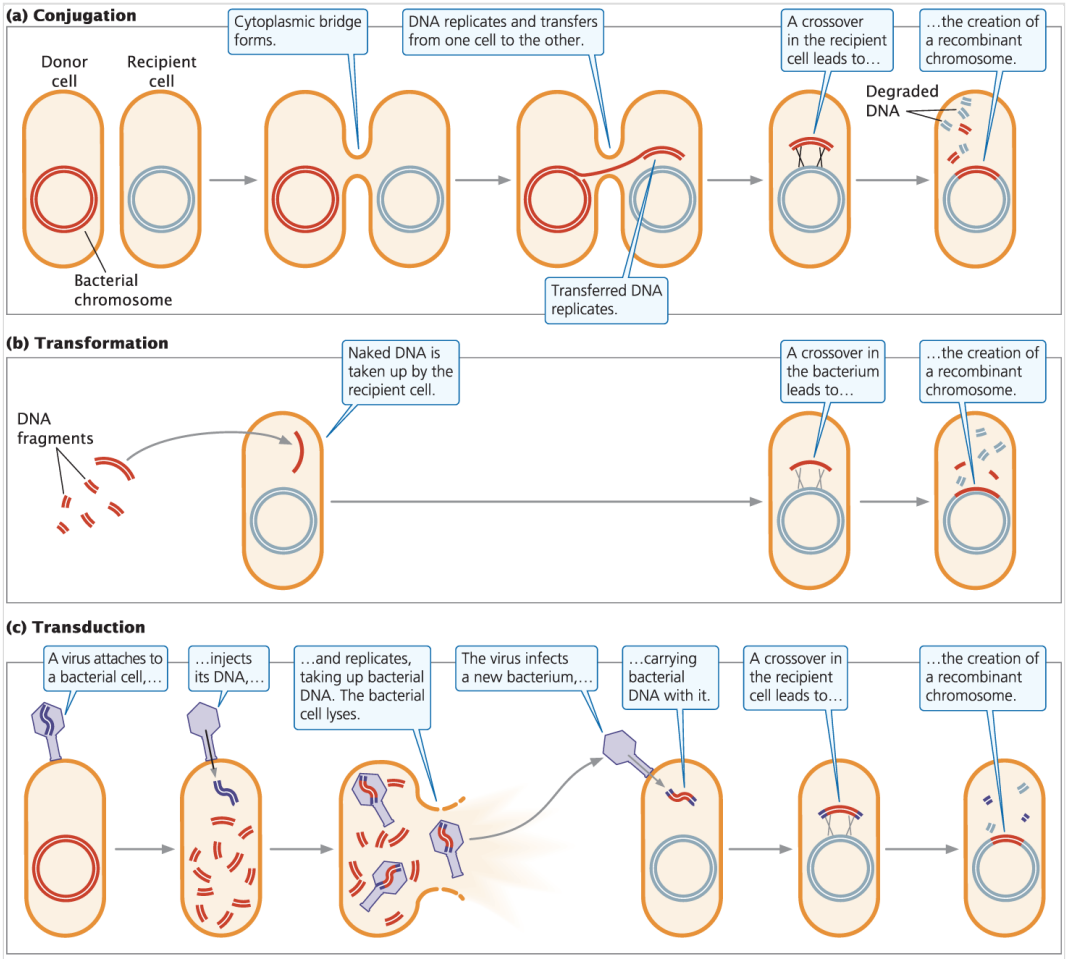
a. They are composed of RNA.
b. They normally exist outside bacterial cells.
c. They possess only a single strand of DNA.
d. They contain an origin of replication.

9.3 Bacteria Exchange Genes Through Conjugation, Transformation, and Transduction

Exchange of genetic information is widespread and common among bacteria and has played an important role in their evolution. Bacteria exchange genetic material by three different mechanisms, all entailing some type of DNA transfer and recombination between the transferred DNA and the bacterial chromosome.

1. **Conjugation** takes place when genetic material passes directly from one bacterium to another (**Figure 9.7a**). In conjugation, two bacteria lie close together and a connection forms between them. A plasmid or a part of the bacterial chromosome passes from one cell (the donor) to the other (the recipient). After conjugation, crossing over may take place between homologous sequences in the transferred DNA and the chromosome of the recipient cell. In conjugation, DNA is transferred only from donor to recipient, with no reciprocal exchange of genetic material.
2. **Transformation** takes place when a bacterium takes up DNA from the medium in which it is growing (**Figure 9.7b**). After transformation, recombination may take place between the introduced genes and those of the bacterial chromosome.
3. **Transduction** takes place when bacterial viruses (bacteriophages) carry DNA from one bacterium to another

(Figure 9.7c). Inside the bacterium, the newly introduced DNA may undergo recombination with the bacterial chromosome.



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9.7 Conjugation, transformation, and transduction are the three processes of gene transfer in bacteria. For the transferred DNA to be stably inherited, all three processes require the transferred DNA to undergo recombination with the bacterial chromosome.

Description

Illustration a at the top shows the process of conjugation. A donor cell with a red bacterial chromosome and a recipient cell with a green chromosome are shown. A forward arrow leads to the fusion of both these cells in the middle section of the cell. A caption reads, Cytoplasmic bridge forms. Another forward arrow leads to the bacterial chromosome that

replicates and migrates to the recipient cell. A caption pointing towards the migrating D N A reads, Transferred D N A replicates. Another caption from the fusion point reads, D N A replicates and transfers from one cell to the other. A forward arrow leads to recipient cell with crossing over occurring between the donor and recipient chromosome. A caption reads, a crossover in the recipient cell leads to the creation of a recombinant chromosome. A forward arrow leads to the recipient cell with a recombinant chromosome and some pieces of degraded D N A.

Illustration b below shows the process of transformation. D N A fragments are present outside the cell. An arrow from one such fragments leads to inside a bacterial cell and a fragment is shown here. A caption reads, Naked D N A is taken up by the recipient cell. Crossing over between the outside D N A fragments and bacterial chromosome occurs. The next picture shows a bacterial cell with recombinant D N A and some smaller D N A fragments. A caption reads, A crossover in the bacterium leads to the creation of a recombinant chromosome.

Illustration c below shows the process of transduction. The process starts with a viral cell attaching to bacterial cell surface. A caption reads, a virus attaches to a bacterial cell, injects its D N A, and replicates taking up bacterial D N A. The bacterial cell lyses. The next picture shows fragments of bacterial D N A along with a fragment of viral D N A inside the bacterium. The bacterial cell is lysed and viral cells take up the bacterial chromosome. This viral cell infects another bacterial cell. A caption reads, The virus infects a new bacterium, carrying bacterial D N A with it. Crossing over between the viral and bacterial D N A occurs. A caption reads, A crossover in the recipient cell leads to the creation of a recombinant chromosome. The last picture shows a bacterial cell recombinant D N A and some fragments of D N A left over.

Not all bacterial species exhibit all three types of DNA transfer. Conjugation takes place more frequently in some species than in others. Transformation takes place to a limited extent in many species of bacteria, but laboratory techniques can increase the rate of DNA uptake. Most bacteriophages have a limited host range, so

transduction normally takes place between bacteria of the same or closely related species only.

These processes of genetic exchange in bacteria differ from diploid eukaryotic sexual reproduction in two important ways. First, DNA exchange and reproduction are not coupled in bacteria; bacteria often undergo reproduction (binary fission) without receiving any DNA from another cell. Second, donated genetic material that is not recombined into the host DNA is usually degraded, so the recipient cell remains haploid. Each type of DNA transfer has been used to map genes.

CONCEPTS

DNA may be transferred between bacterial cells through conjugation, transformation, or transduction. Each type of DNA transfer consists of a one-way movement of genetic information to the recipient cell, sometimes followed by recombination. These processes are not connected to reproduction in bacteria.

✔ CONCEPT CHECK 2

Which process of DNA transfer in bacteria requires a virus?

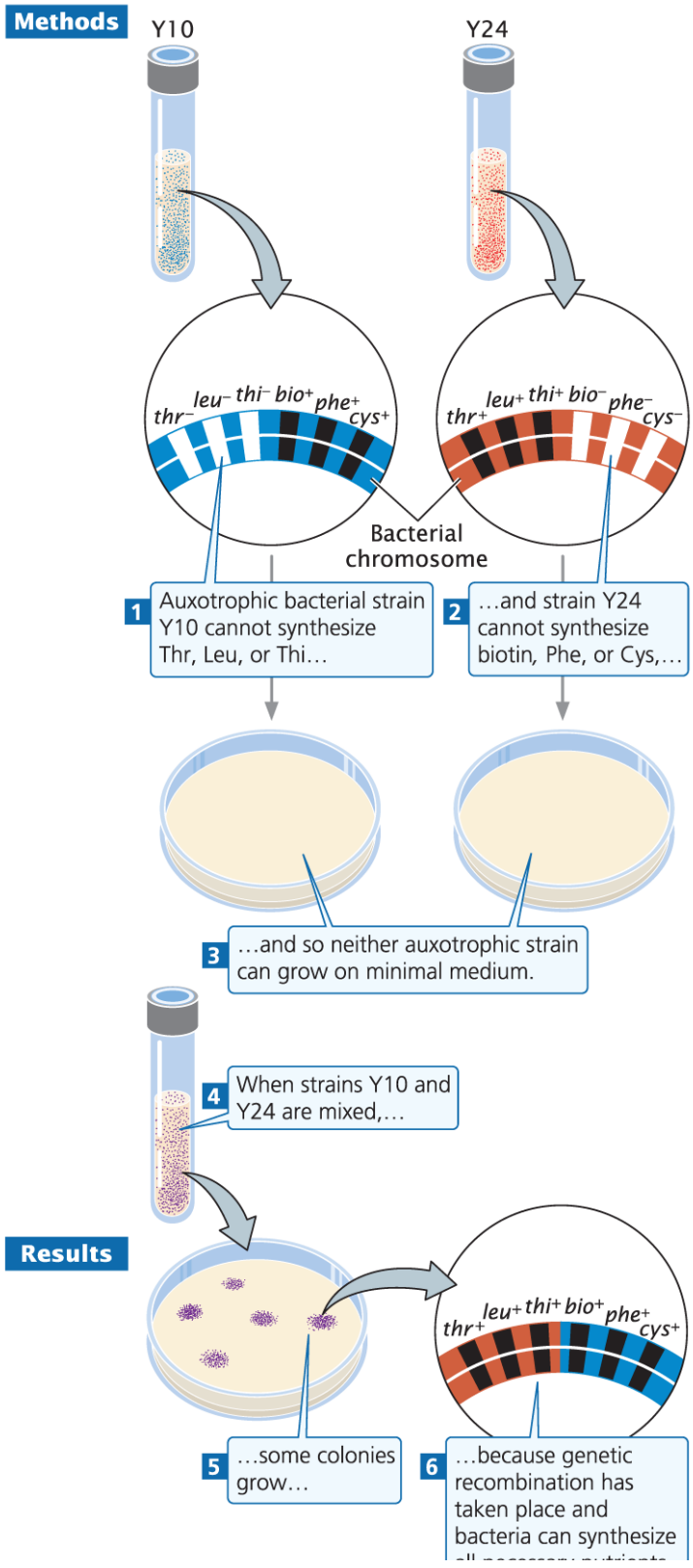
a. Conjugation
b. Transduction
c. Transformation
d. All of the above

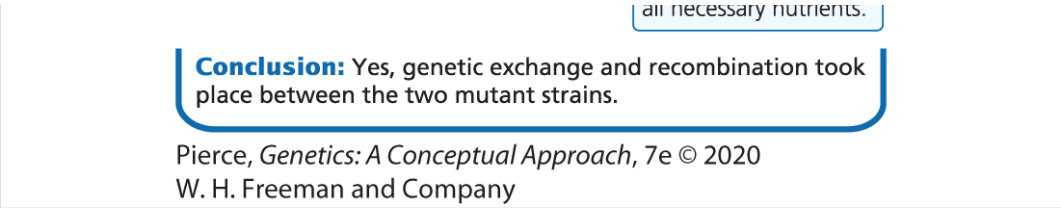
Conjugation

In 1946, Joshua Lederberg and Edward Tatum demonstrated that bacteria can transfer and recombine genetic information, paving the way for the use of bacteria in genetic studies. In the course of their research, Lederberg and Tatum studied auxotrophic strains of *E. coli*. The Y10 strain required the amino acids threonine (and was thus genotypically thr^-) and leucine (leu^-) and the vitamin thiamine (thi^-) for growth, but it did not require the vitamin biotin (bio^+) or the amino acids phenylalanine (phe^+) and cysteine (cys^+); the genotype of this strain can be written as $thr^-\ leu^-\ thi^-\ bio^+\ phe^+\ cys^+$. The Y24 strain had the opposite set of alleles: it required biotin, phenylalanine, and cysteine in its medium, but it did not require threonine, leucine, or thiamine; its genotype was $thr^+\ leu^+\ thi^+\ bio^-\ phe^-\ cys^-$. In one experiment, Lederberg and Tatum mixed Y10 and Y24 bacteria together and plated them on a minimal medium (**Figure 9.8**). Each strain was also plated separately on a minimal medium.

Experiment

Question: Do bacteria exchange genetic information?





9.8 Lederberg and Tatum's experiment demonstrated that bacteria undergo genetic exchange.

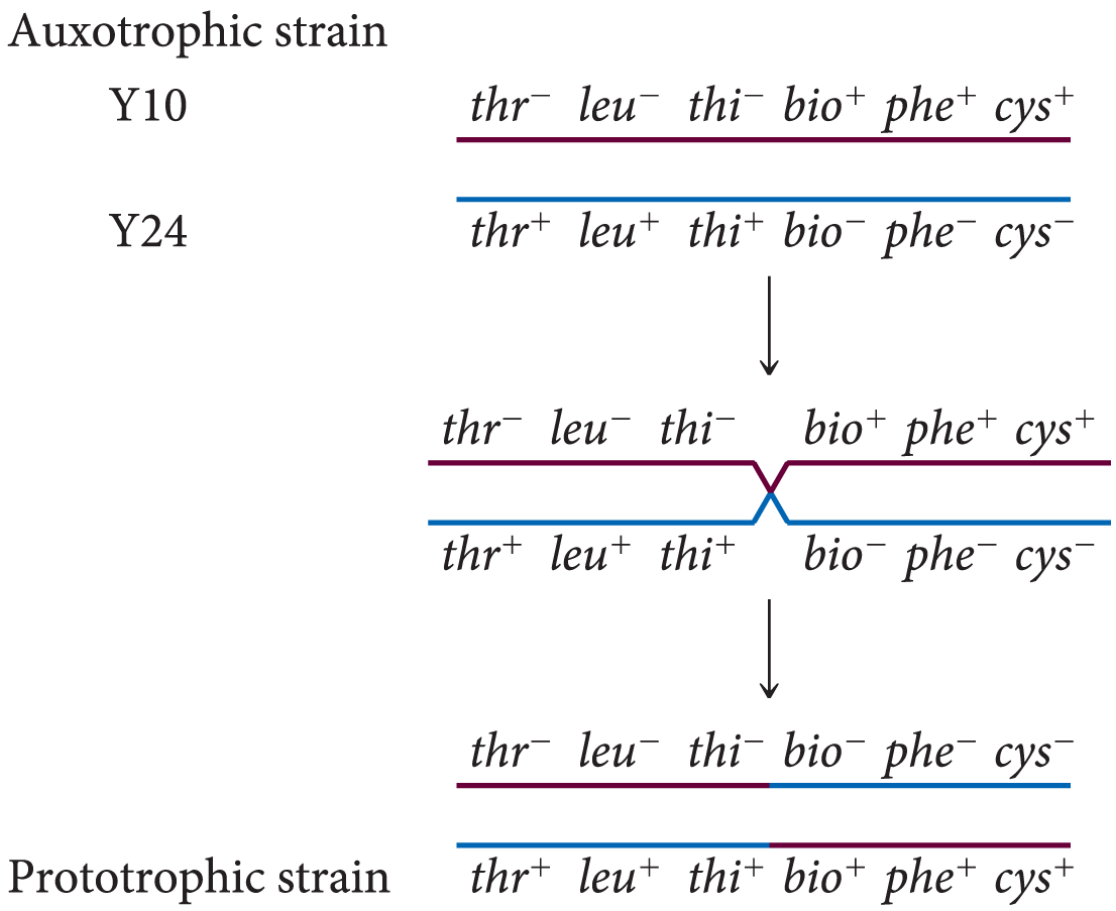
Description

The illustration shows question for the experiment: Do bacteria exchange genetic information? The methods below show the Y 10 and Y 24 strains in two different test tubes and a zoomed in image of their bacterial chromosomes with genes involved. Functional genes are represented by black color and non-functional genes by white color. A picture of petriplate with no colony is present below each strain. Three captions pointing towards the bacterial chromosome of Y 10 strain, strain Y 24, and the petriplates read, auxotrophic bacterial strain Y 10 cannot synthesize Thr, Leu, or t h i and strain Y 24 cannot synthesize biotin, phe, or cys, and so neither auxotrophic strain can grow on minimal medium. The results below start with pictures of a test tube of bacteria, a petri plate with colonies, and a zoomed in image of the bacterial chromosome with all of the genes blacked in. Three captions pointing towards the test tube, a bacterial colony, and the bacterial chromosome read, When strains Y 10 and Y 24 are mixed, some colonies grow because genetic recombination has taken place and bacteria can synthesize all necessary nutrients. A caption below reads, Conclusion: Yes, genetic exchange and recombination took place between the two mutant strains.

Alone, neither Y10 nor Y24 grew on a minimal medium: each strain required nutrients that were absent. Strain Y10 was unable to grow because it required threonine, leucine, and thiamine, which were absent in the minimal medium; strain Y24 was unable to grow because it required biotin, phenylalanine, and cysteine, which were also absent from the minimal medium. When Lederberg and Tatum mixed the two strains, however, a few colonies did grow on the

minimal medium. These prototrophic bacteria must have had genotype thr^+ leu^+ thi^+ bio^+ phe^+ cys^+. Where had they come from?

If mutations were responsible for the prototrophic colonies, then some colonies should also have grown on the plates containing Y10 or Y24 alone, but no bacteria grew on those plates. Multiple simultaneous mutations ($thr^- \rightarrow thr^+$, $leu^- \rightarrow leu^+$, and $thi^- \rightarrow thi^+$ in strain Y10 or $bio^- \rightarrow bio^+$, $phe^- \rightarrow phe^+$, and $cys^- \rightarrow cys^+$ in strain Y24) would have been required for either strain to become prototrophic by mutation, which was very improbable. Lederberg and Tatum concluded that some type of genetic transfer and recombination had taken place:

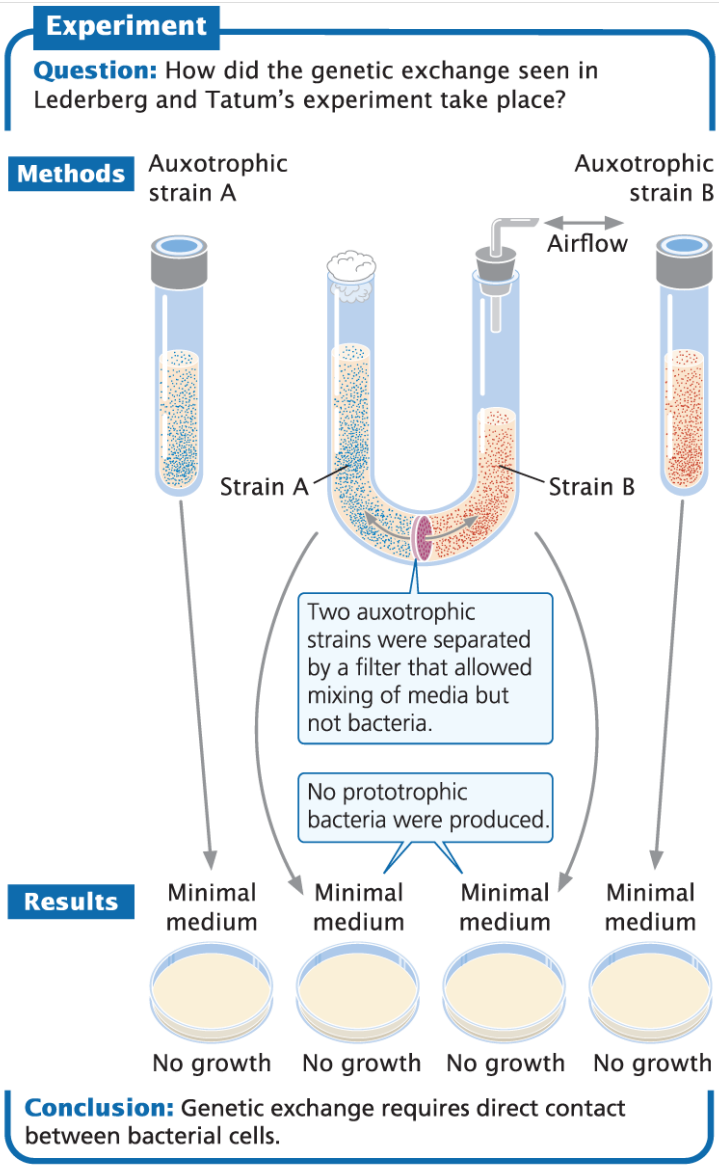


Description

The genes present on the Y 10 strain are t h r superscript negative, l e u superscript negative, and t h i superscript negative and b i o superscript positive, p h e superscript positive, and c y s superscript positive. The genes present on the Y 24 strain are t h r superscript positive, l e u superscript positive, and t h i superscript positive and b i o superscript negative, p h e superscript negative, and c y s superscript negative. The next pair of lines show a crossover event in the middle. An arrow pointing down shows the results of the recombination. The strand above is all negative and the other strand below is all positive with same gene sequence and labeled a prototrophic strain.

What they did not know was *how* it had taken place.

To study this problem, Bernard Davis constructed a U-shaped tube (Figure 9.9) that was divided into two compartments by a filter with fine pores. This filter allowed a liquid medium to pass from one side of the tube to the other, but the pores of the filter were too small to allow the passage of bacteria. Two auxotrophic strains of bacteria were placed on opposite sides of the filter, and suction was applied alternately to the ends of the U-tube, causing the medium to flow back and forth between the two compartments. Despite hours of incubation in the U-tube, bacteria plated on the minimal medium did not grow; there had been no genetic exchange between the strains. The exchange of bacterial genes clearly required direct contact, or conjugation, between the bacterial cells.



9.9 Davis's U-tube experiment.

Description

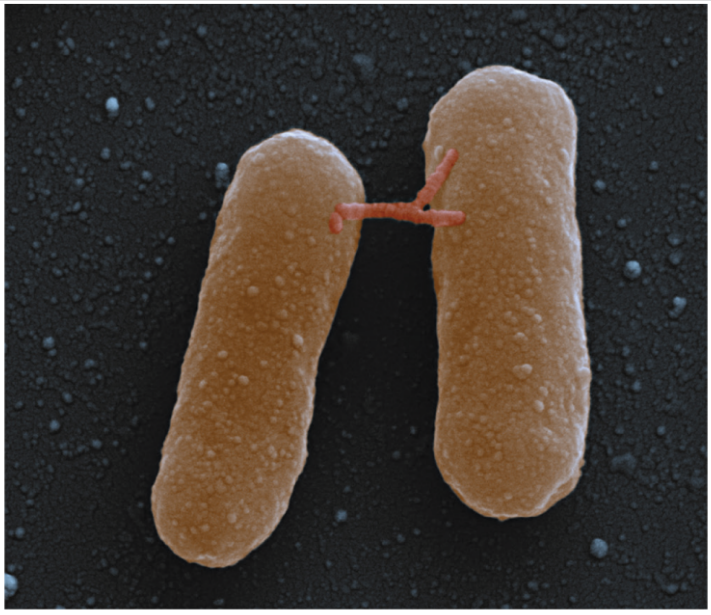
The illustration shows question as follows: How did the genetic exchange seen in Lederberg and Tatum's experiment take place? The methods below show two auxotrophic strains A and B. Both of these strains are put into a U-shaped tube that has a filter in between physically separating the two bacteria. A caption pointing towards the filter reads, Two auxotrophic strains were separated by a filter that allowed mixing of medium but not bacteria. The results below show four petriplates having minimal media. Bacteria in the

first petriplate are cultured directly from the test tube containing auxotrophic strain A. Bacteria in the second petriplate are cultured from strain A in the U-shaped tube. Bacteria in the third petriplate are cultured from strain B in the U-shaped tube. All the four petriplates show no growth. A caption below reads, Conclusion: Genetic exchange requires direct contact between bacterial cells.

F^+ AND F^- CELLS

In many bacterial species, conjugation depends on a plasmid or DNA element that is present in the donor cell and absent in the recipient cell. In *E. coli*, this plasmid is known as the fertility (F) factor. Cells that contain the F factor are referred to as F^+, and cells lacking the F factor are F^-.

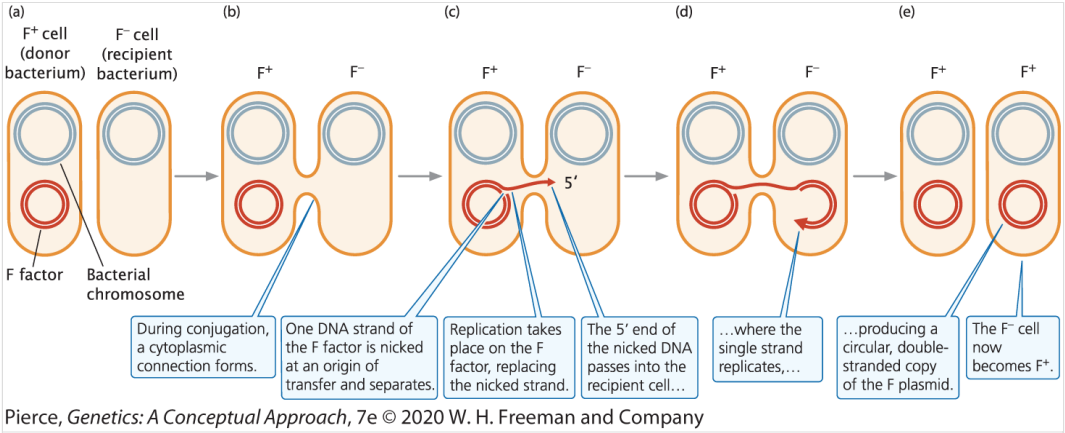
The F factor is an episome that contains an origin of replication and a number of genes required for conjugation (see Figure 9.6). For example, some of the genes in this plasmid encode sex **pili** (singular, pilus), slender extensions of the cell membrane. A cell containing the F factor produces sex pili, one of which makes contact with a receptor on an F^- cell (**Figure 9.10**) and pulls the two cells together. DNA is then transferred from the F^+ cell to the F^- cell. Conjugation can take place only between a cell that possesses the F factor and a cell that lacks the F factor.



Eye of Science/Science Source.

9.10 Sex pili connect F^+ and F^- cells during bacterial conjugation.

In most cases, the only genes transferred during conjugation between an F^+ and an F^- cell are those on the F factor (**Figure 9.11a** and b). Transfer is initiated when one of the DNA strands on the F factor is nicked at an origin of transfer (*oriT*). One end of the nicked DNA separates from the circular F plasmid and passes into the recipient cell (**Figure 9.11c**). Replication takes place on the F factor, proceeding around the circular plasmid in the F^+ cell and replacing the transferred strand. Because the F factor in the F^+ donor cell is always nicked at the *oriT* site, this site always enters the recipient cell first, followed by the rest of the plasmid. Thus, the transfer of genetic material has a defined direction. Inside the recipient cell, the single strand replicates, producing a circular, double-stranded copy of the F plasmid (**Figure 9.11d** and e). If the entire F factor is transferred to the recipient F^- cell, that cell becomes an F^+ cell.



9.11 The F factor is transferred during conjugation between an F^+ and an F^- cell.

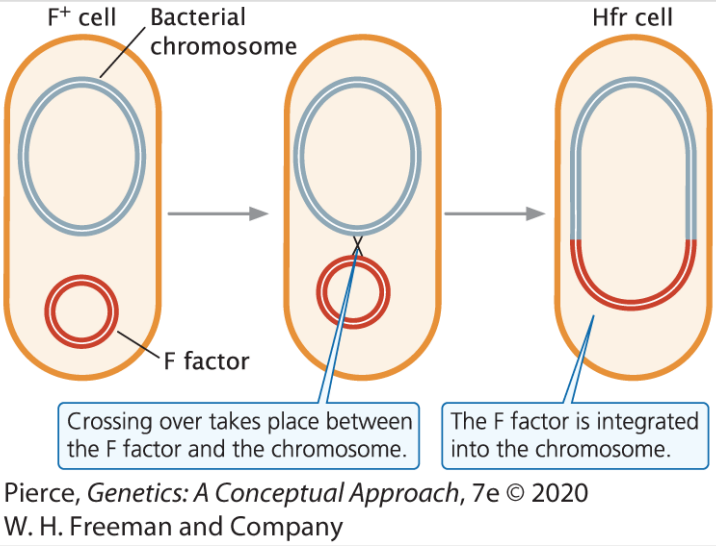
Description

The illustration shows five pictures of bacterial cells labeled a through e. Picture a at the left shows an F superscript positive donor cell and an F superscript negative recipient cell. Both cells have a double stranded bacterial chromosome, but only the F superscript positive cell has a red, double-stranded circular D N A complex labeled F factor. The captions in the illustration describe how the red D N A complex migrates from donor to recipient. The captions read as follows: During conjugation, a cytoplasmic connection forms. One D N A strand of the F factor is nicked at an origin and separates. Replication takes place on the F factor, replacing the nicked strand. The 5 prime end of the nicked D N A passes into the recipient cell where the single strand replicates, producing a circular, double-stranded copy of the F factor. The F superscript negative cell now becomes F superscript positive.

Hfr CELLS

Conjugation transfers genetic material in the F plasmid from F^+ to F^- cells, but it does not account for the transfer of chromosomal genes observed by Lederberg and Tatum. In Hfr (high-frequency

recombination) bacterial strains, the F factor is integrated into the bacterial chromosome (Figure 9.12). Hfr cells behave like F^+ cells, forming sex pili and undergoing conjugation with F^- cells.



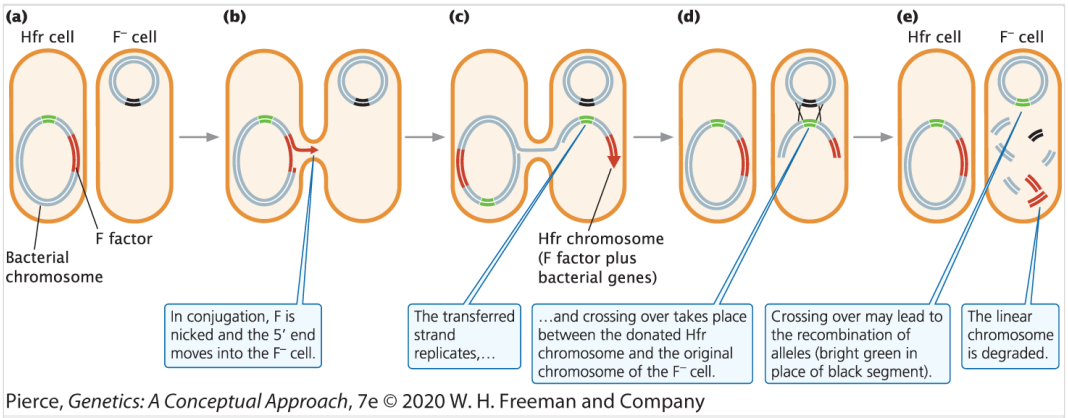
9.12 The F factor is integrated into the bacterial chromosome in an Hfr cell.

Description

The F superscript positive cell has a circular bacterial chromosome and an relatively smaller, circular red F factor plasmid. Crossing over occurs between the chromosome and the F factor. A caption reads, Crossing over takes place between the F factor and chromosome. The cell shows a continuous chromosome with both the F factor and bacterial chromosome fused together and the cell is labeled as H f r cell. Another caption reads, The F factor is integrated into the chromosome.

In conjugation between Hfr and F^- cells (Figure 9.13a), the integrated F factor is nicked, and the end of the nicked strand moves into the F^- cell (Figure 9.13b), just as it does in conjugation between F^+ and F^- cells. But because in an Hfr cell, the F factor has been integrated into the bacterial chromosome, the chromosome follows

the F factor into the recipient cell. How much of the bacterial chromosome is transferred depends on the length of time that the two cells remain in conjugation.



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9.13 Bacterial genes may be transferred from an Hfr cell to an F⁻ cell in conjugation.

Description

The illustration a shows an H f r cell and an F superscript negative cell. The H f r cell has a bacterial chromosome including the regular genome in gray, the F factor portion in red, and one stretch of the chromosome is bright green. The F superscript negative cell next to it has a gray genome with a small double-stranded portion that is black. The illustration a leads to next illustration. In the illustration b, the bacterial cells are fused in between and the red portion of the strand is nicked and starts to begin the transfer. A caption reads, In conjugation, F is nicked and the 5 prime end moves into the F superscript negative cell. In the illustration c, the transferred strand starts the replication and a caption reads, The transferred strand replicates, and crossing over takes place between the donated H f r chromosome and the original chromosome of the F superscript negative cell. In the illustration d, the bridge between the two bacterial cells is not present and crossing over is taking place in the F superscript negative cell. The illustration e shows two cells as before, H f r cell and F superscript negative cell. H f r cell is exactly the same, but the F superscript negative cell has a green portion in its genome with some degraded fragments of gray, red, and black color. Captions read, Crossing over may lead to the recombination of alleles, or bright green in place of black segment. The linear chromosome is degraded.

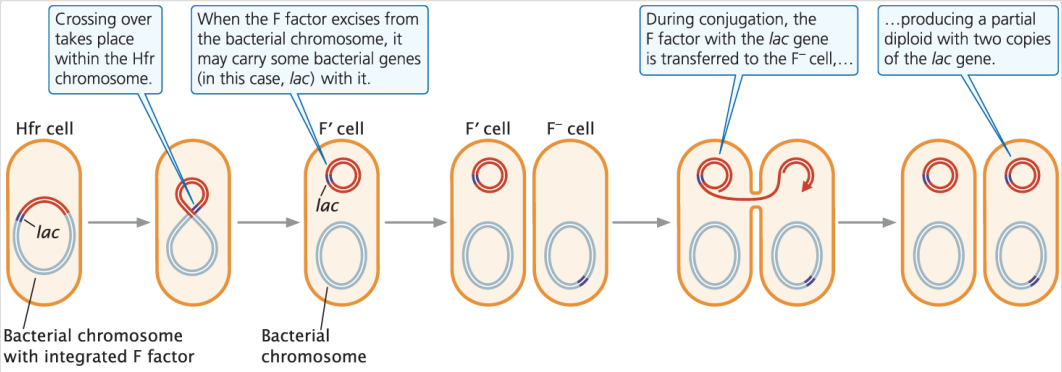
Inside the recipient cell, the donor DNA strand replicates (Figure 9.13c), and crossing over between it and the original chromosome of the F^- cell (Figure 9.13d) may take place. This chromosomal gene transfer between Hfr and F^- cells explains how the recombinant prototrophic cells observed by Lederberg and Tatum were produced. After crossing over has taken place in the recipient cell, the donated strand is degraded and the recombinant recipient chromosome remains (Figure 9.13e), to be replicated and passed on to later generations by binary fission.

In a mating between an Hfr and an F^- cell, the F^- cell almost never becomes F^+ or Hfr because the F factor is nicked in the middle at the initiation of strand transfer, which places part of the F factor at the beginning and part at the end of the strand that is transferred. To become F^+ or Hfr, the recipient cell must receive the entire F factor, which requires that the entire donor chromosome be transferred. This event happens rarely because most conjugating cells break apart before the entire chromosome has been transferred.

The F plasmid in an F^+ cell integrates into the bacterial chromosome, causing the F^+ cell to become Hfr, at a frequency of only about 1 in 10,000. This low frequency accounts for the low rate of recombination observed by Lederberg and Tatum in their F^+ cells. The F factor is excised from the bacterial chromosome at a similarly low rate, causing a few Hfr cells to become F^+.

F′ CELLS

When an F factor is excised from the bacterial chromosome, a small amount of the bacterial chromosome may be removed with it, and these chromosomal genes will then be carried with the F plasmid (Figure 9.14). Cells containing an F plasmid with some bacterial genes are called F prime (F′) cells. For example, if an F factor integrates into a chromosome at a position adjacent to the *lac* genes (genes that enable a cell to metabolize the sugar lactose), the F factor may pick up *lac* genes when it is excised, becoming F′*lac*. F′ cells can conjugate with F⁻ cells because F′ cells possess the F plasmid, with all the genetic information necessary for conjugation and DNA transfer. Characteristics of different mating types of *E. coli* (cells with different types of F) are summarized in Table 9.2.



9.14 An Hfr cell may be converted into an F′ cell when the F factor is excised from the bacterial chromosome and carries bacterial genes with it.

Conjugation between an F′ cell and an F⁻ cell produces a partial diploid.

Description

The illustration starts with an H f r cell having a circular bacterial chromosome with integrated F factor and l a c gene. Crossing over occurs within the chromosome and F prime cell is produced with two circular D N A; one is the bacterial chromosome and the

other have F factor and l a c gene. The captions read as follows: Crossing over takes place within the H f r chromosome. When the F factor is excised from the bacterial chromosome, it may carry some chromosomal genes, in this case, l a c, with it. This leads to two cells; one with the F factor and l a c gene and bacterial chromosome labeled F prime cell and other with only bacterial chromosome labeled F superscript negative cell. The F factor transfers from the F prime to the F superscript negative cell. Both the cells have F factor and l a c gene now. The captions here read: During conjugation, the F factor with the l a c gene is transferred to the F superscript negative cell, producing a partial diploid with 2 copies of the l a c gene.

TABLE 9.2 Characteristics of *E. coli* cells with different types of F factor

Type	F Factor Characteristics	Role in Conjugation
F^+	Present as separate circular plasmid	Donor
F^-	Absent	Recipient
Hfr	Present, integrated into bacterial chromosome	High-frequency donor
F'	Present as separate circular plasmid, carrying some bacterial genes	Donor

During conjugation between an F' cell and an F^- cell, the F plasmid is transferred to the F^- cell, which means that any genes on the F plasmid, including those from the bacterial chromosome, may be transferred to the F^- recipient cell (see Figure 9.14). This process produces partial diploids, or merozygotes, which are cells with two copies of some genes, one on the bacterial chromosome and one on the newly introduced F plasmid. The outcomes of conjugation between different mating types of *E. coli* are summarized in **Table 9.3**.

TABLE 9.3 Results of conjugation between *E. coli* cells with different F factor

Conjugating Cells	Cell Types Present after Conjugation
$F^+ \times F^-$	Two F^+ cells (F^- cell becomes F^+)
Hfr × F^-	One Hfr cell and one F^- cell (no change)*
$F' \times F^-$	Two F' cells (F^- cell becomes F')

*Rarely, the F^- cell becomes F^+ in an Hfr × F^- conjugation if the entire chromosome is transferred during conjugation.

CONCEPTS

Conjugation in *E. coli* is controlled by an episome called the F factor. Cells containing the F factor (F^+ cells) are donors of DNA; cells lacking the F factor (F^- cells) are recipients. In Hfr cells, the F factor is integrated into the bacterial chromosome; these cells donate DNA to F^- cells at a high frequency. F' cells contain a copy of the F plasmid with some bacterial genes.

✔ CONCEPT CHECK 3

Conjugation between an F^+ and an F^- cell usually results in

a. two F^+ cells.
b. two F^- cells.
c. an F^+ and an F^- cell.
d. an Hfr cell and an F^+ cell.

MAPPING BACTERIAL GENES WITH INTERRUPTED CONJUGATION

The transfer of DNA that takes place during conjugation between Hfr and F^- cells has been used to map bacterial genes, although it is not commonly employed today. (Most genes in bacteria are mapped today using DNA sequencing; see Chapter 19.) Conjugation mapping used conjugation between Hfr cells and F^- cells. Transfer of the entire *E. coli* chromosome from the Hfr donor to the F^- recipient requires about 100 minutes; if conjugation is interrupted before 100 minutes have elapsed, only part of the donor chromosome will have passed into the F^- cell and had an opportunity to recombine with the recipient chromosome. Chromosome transfer always begins within the integrated F factor and proceeds in a defined direction, so genes are transferred according to their sequence on the chromosome. The times required for individual genes to be transferred indicate their relative positions on the chromosome. View (A) **Animation 9.1** to see how genes are mapped using interrupted conjugation.

CONCEPTS

Conjugation can be used to map bacterial genes by mixing Hfr and F^- cells of different genotypes and interrupting conjugation at regular intervals. The amounts of time required for individual genes to be transferred from the Hfr to the F^- cells indicate the relative positions of the genes on the bacterial chromosome.

✔ CONCEPT CHECK 4

Interrupted conjugation was used to map three genes in *E. coli*. The donor genes first appeared in the recipient cells at the following times: *gal*, 10 minutes; *his*, 8 minutes; *pro*, 15 minutes. Which gene is in the middle?

Natural Gene Transfer and Antibiotic Resistance

Antibiotics are substances that kill bacteria. Their development and widespread use have greatly reduced the threat of infectious disease and saved countless lives. But many pathogenic bacteria have developed resistance to antibiotics, particularly in environments where antibiotics are routinely used, such as hospitals, livestock operations, and fish farms. In these environments, where antibiotics are continually present, the only bacteria to survive are those that possess antibiotic resistance. No longer in competition with other bacteria, resistant bacteria multiply quickly and spread. In this way, the presence of antibiotics selects for resistant bacteria and reduces the effectiveness of antibiotic treatment for infections.

Antibiotic resistance in bacteria frequently results from the action of genes located on *R plasmids,* small circular plasmids that can be transferred by conjugation. Some drug-resistant R plasmids convey resistance to several antibiotics simultaneously. Ironic but plausible sources of some of the resistance genes found in R plasmids are the microbes that produce antibiotics in the first place.

Widespread use of antibiotics not only increases the frequency of resistant pathogenic bacteria it also affects nonpathogenic bacteria. The community of bacteria associated with humans—in our mouth, in our gut, and on our skin—is collectively referred to as the **human**

microbiome. Much research indicates that the microbiome is essential to human digestion, pathogen resistance, weight control, and physiology. It is clear that in developed countries, the human microbiome has changed significantly over the past hundred years, probably as a result of widespread antibiotics but perhaps also due to cleaner and chlorinated drinking water, better sanitation, and use of infant formula. Some studies have suggested that modern societies may have lost more than half of their microbial diversity. What effect this erosion of our microbiome will have on our health and function isn't yet clear.

THINK-PAIR-SHARE Question 3

Transformation in Bacteria

A second way in which DNA can be transferred between bacteria is through transformation (see Figure 9.7b). Transformation played an important role in the initial identification of DNA as the genetic material, as we will see in Chapter 10.

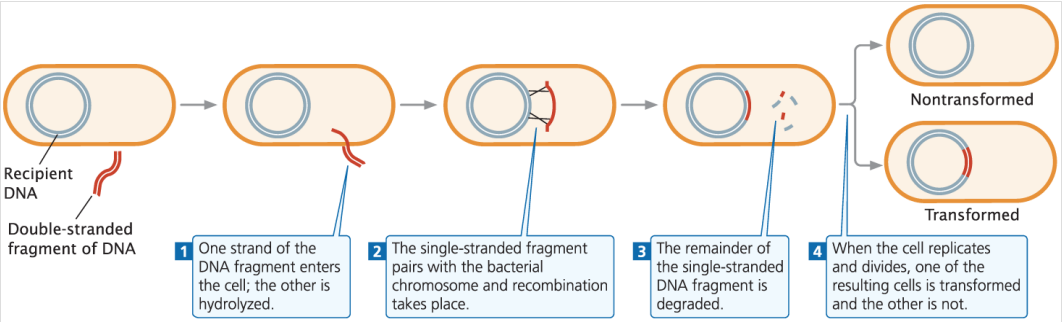
Transformation requires both the uptake of DNA from the surrounding medium and its incorporation into a bacterial chromosome or a plasmid. It may occur naturally when dead bacteria break down and release DNA fragments into the environment. In soil and marine environments, transformation may

be an important route of genetic exchange for some bacteria. Transformation is also an important technique for transferring genes to bacteria in the laboratory.

MECHANISM OF TRANSFORMATION

Cells that can take up DNA through their cell membranes are said to be **competent**. Some species of bacteria take up DNA more easily than others: competence is influenced by growth stage, the concentration of available DNA in the environment, and other environmental factors. The DNA that a competent cell takes up need not be bacterial: virtually any type of DNA (bacterial or otherwise) can be taken up by competent cells under the appropriate conditions.

As a DNA fragment enters the cell in the course of transformation (**Figure 9.15**), one of the strands is broken up, whereas the other strand moves across the membrane and may pair with a homologous region and become integrated into the bacterial chromosome. Its integration into the recipient chromosome requires two crossover events, after which the remaining single-stranded DNA is degraded by bacterial enzymes. In some bacteria, double-stranded DNA moves across the cell membrane and is integrated into the bacterial chromosome. Cells that receive genetic material through transformation are called **transformants**.



9.15 Genes can be transferred between bacteria through transformation.

Description

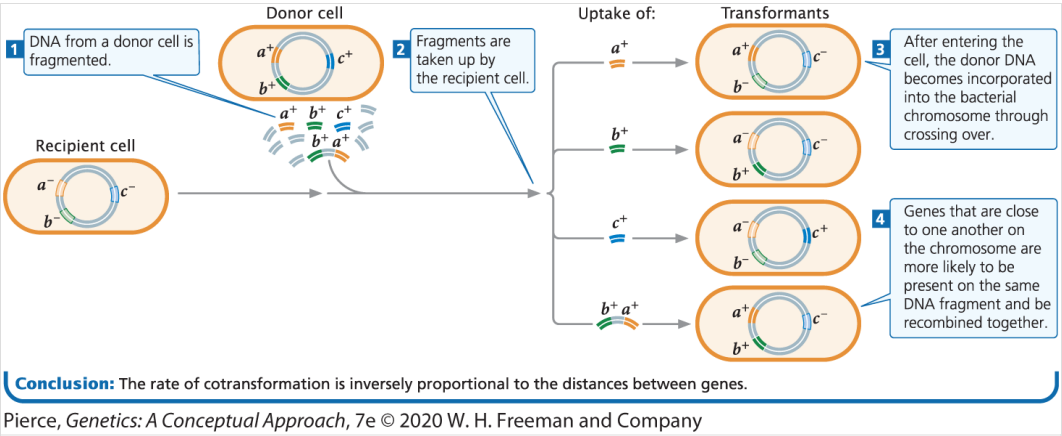
The illustration shows a bacterial cell on the left side with a naked doubled-stranded D N A from the outside integrating into the cell. Only a single strand red in color enters the cell. A caption reads, One strand of the D N A fragment enters the cell; the other is hydrolyzed. Crossing over occurs between the recipient D N A and the single-stranded fragment. A caption reads, The single-stranded fragment pairs with the bacterial chromosome and recombination takes place. Now the bacterial cell has the single-stranded D N A incorporated into one of its strands of recipient D N A. Some fragments of both the outside D N A and the bacterial D N A are present in the cell. A caption reads, The remainder of the single-stranded D N A fragment is degraded. Cell division occurs and two daughter cells are produced. One has only the bacterial chromosome and is labeled non-transformed, while the other has outside D N A incorporated into its genome and is labeled transformed. A caption reads, When the cell replicates and divides, one of the resulting cells is transformed and the other is not.

Bacterial geneticists have developed techniques for increasing the frequency of transformation in the laboratory to introduce particular DNA fragments or whole plasmids into cells. They have also developed strains of bacteria that are more competent than wild-type cells. Treatment with calcium chloride, heat shock, or an electrical field makes bacterial membranes more porous and permeable to DNA. The efficiency of transformation can also be

increased by using high concentrations of DNA. These techniques enable researchers to transform bacteria such as *E. coli*, which are not naturally competent.

GENE MAPPING WITH TRANSFORMATION

Transformation, like conjugation, has been used in the past to map bacterial genes. Transformation mapping requires two strains of bacteria that differ in several genetic traits; for example, the recipient strain might be $a^- b^- c^-$ (auxotrophic for three nutrients), and the donor strain might be $a^+ b^+ c^+$ (prototrophic for the same three nutrients) (Figure 9.16). DNA from the donor strain is isolated, purified, and fragmented. The recipient strain is treated to increase its competence, and DNA from the donor strain is added to the medium. Fragments of the donor DNA enter the recipient cells and undergo recombination with homologous DNA sequences on the bacterial chromosome.



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9.16 Transformation can be used to map bacterial genes.

Description
The illustration shows a recipient with a chromosome that is negative for genes a, b, and c. The donor cell is positive for genes a, b, and c. D N A fragments and genes positive for a, b, and c are also present outside the donor cell. A caption reads, D N A from a donor cell is fragmented. A second caption reads, Fragments are taken up by the recipient cell. An arrow from the recipient cell points to 4 different types of transformants for the uptake of a superscript positive, b superscript positive, c superscript positive, and b superscript positive a superscript positive respectively: First transformant has a superscript positive, b superscript negative, and c superscript negative genes. A caption reads, After entering the cell, the donor D N A becomes incorporated into the bacterial chromosome through crossing over. The second transformant has a superscript negative, b superscript positive, and c superscript negative genes. The third transformant has a superscript negative, b superscript negative, and c superscript positive genes. The fourth transformant has a superscript positive, b superscript positive, and c superscript negative genes. A caption reads, Genes that are close to one another on the chromosome are more likely to be present on the same D N A fragment and be recombined together. A caption below reads, Conclusion: The rate of cotransformation is inversely proportional to the distances between genes.

Bacterial genes can be mapped by observing the rate at which two or more genes are transferred to the recipient chromosome together, or **cotransformed**. When the donor DNA is fragmented before transformation, genes that are physically closer together on the bacterial chromosome are more likely to be present on the same DNA fragment and transferred together, as shown for genes a^+ and b^+ in Figure 9.16. Genes that are far apart are unlikely to be present on the same DNA fragment and are rarely cotransformed. Therefore, the frequency of cotransformation can be used to map bacterial genes. If genes *a* and *b* as well as genes *b* and *c* are frequently cotransformed, but genes *a* and *c* are rarely cotransformed, then

gene *b* must be between *a* and *c*—the gene order is *a*, *b*, *c*. **› TRY PROBLEM 23**

CONCEPTS

Genes can be mapped in bacteria by taking advantage of transformation—the ability of bacteria to take up DNA from the environment and incorporate it into their chromosomes through crossing over. The relative rates at which pairs of genes are cotransformed indicate the distance between them: the higher the rate of cotransformation, the closer the genes are on the bacterial chromosome.

✔ CONCEPT CHECK 5

A bacterial strain with genotype his^- leu^- thr^- is transformed with DNA from a strain that is his^+ leu^+ thr^+. A few leu^+ thr^+ cells and a few his^+ thr^+ cells are found, but no his^+ leu^+ cells are observed. Which genes are farthest apart?

Bacterial Genome Sequences

Genetic maps serve as the foundation for the more detailed information provided by DNA sequencing, such as gene content and organization (see Chapter 19 for a discussion of gene sequencing). Geneticists have now determined the complete nucleotide sequences of more than two thousand bacterial genomes (see Table 20.3), and many additional microbial sequencing projects are under way.

Most bacterial genomes contain from 1 million to 4 million base pairs of DNA, but a few are much smaller (e.g., 580,000 bp in *Mycoplasma genitalium*) and some are considerably larger (e.g., more than 7 million bp in *Mesorhizobium loti*). The small size of bacterial genomes (relative to those found in multicellular eukaryotes, which often have billions of base pairs of DNA) is thought to be an adaptation for rapid cell division, because the rate of cell division is limited by the time required to replicate the DNA. On the other hand, the lack of mobility in most bacteria requires metabolic and environmental flexibility, so genome size and content are likely to reflect a balance between the opposing evolutionary forces of gene loss to maintain rapid reproduction and gene acquisition to ensure flexibility.

The functions of a substantial proportion of genes in all bacteria have not been determined. Certain genes, particularly those with related functions, tend to reside next to one another, but these clusters are in very different locations in different species, suggesting that bacterial genomes are constantly being reshuffled. Comparisons of the gene sequences of pathogenic and benign bacteria are helping identify genes implicated in disease and may suggest new targets for antibiotics and other antimicrobial agents.

Horizontal Gene Transfer

The availability of bacterial genome sequences has provided evidence that many bacteria have acquired genetic information from other species of bacteria—and sometimes even from eukaryotic organisms—in a process called **horizontal gene transfer**. In most eukaryotes, genes are passed only among members of the same species through reproduction (a process called vertical transmission); that is, genes are passed from one generation to the next. In horizontal gene transfer, genes can be passed between individual members of different species by nonreproductive mechanisms, such as conjugation, transformation, and transduction. Evidence suggests that horizontal gene transfer has taken place repeatedly among bacteria. For example, as much as 17% of *E. coli*'s genome has been acquired from other bacteria through horizontal gene transfer. Of medical significance, some pathogenic bacteria have acquired the genes necessary for infection, whereas others have acquired genes that confer resistance to antibiotics.

Because of the widespread occurrence of horizontal gene transfer, many bacterial chromosomes are a mixture of genes inherited through vertical transmission and genes acquired through horizontal gene transfer. This observation has caused some biologists to question whether the species concept is appropriate for bacteria. A *species* is often defined as a group of organisms that are reproductively isolated from other groups, have a set of genes in common, and evolve together (see Chapter 26). Because of horizontal gene transfer, the genes of one bacterial species are not

isolated from the genes of other species, making the traditional species concept difficult to apply. Horizontal gene transfer also muddies the determination of the ancestral relationships among bacteria. The reconstruction of ancestral relationships is usually based on genetic similarities and differences: organisms that are genetically similar are assumed to have descended from a recent common ancestor, whereas organisms that are genetically distinct are assumed to be more distantly related. Through horizontal gene transfer, however, even distantly related bacteria may share some genes and thus appear to have descended from a recent common ancestor. The nature of species and how to classify bacteria are currently controversial topics within the field of microbiology.

9.4 Bacterial Defense Mechanisms

As discussed earlier, we live in a microbial world. Bacteria and archaea are found in almost every conceivable environment, often in very large numbers, and thrive where other organisms perish. In spite of their abundance, being a microbe is not easy. Bacteria and archaea face significant challenges, the most serious of which are viruses. Scientists have estimated that there are ten times as many viruses as bacteria and archaea, so viruses are a constant threat. Not surprisingly, bacteria and archaea have evolved a number of mechanisms to prevent the entry and reproduction of viruses and other forms invading DNA.

To invade a cell, a virus must first attach to the cell wall or membrane and inject its DNA or RNA into the cell. Many bacteria and archaea have evolved mechanisms to prevent viral attachment and injection of the viral DNA or RNA into the cell. Some bacteria turn off the expression of receptors to which viruses attach; other bacteria secrete polysaccharide coats that limit the access of viruses to those receptors. Bacteria can also produce proteins on the cell membrane that prevent viruses from injecting their nucleic acids into the cell. In other cases, a bacterium that has been infected self-destructs, so that the virus within it is unable to replicate and spread to other bacteria. Other defense mechanisms block virus replication.

Restriction-Modification Systems

Another type of bacterial defense consists of **restriction-modification systems**. Bacteria produce enzymes called restriction endonucleases that recognize and cleave double-stranded DNA at specific nucleotide sequences (see Chapter 19). These enzymes cut up viral DNA, destroying the viral genome before it can replicate. Bacteria protect their own DNA from cleavage by modifying the recognition sequences on their DNA, usually by the addition of methyl groups. Restriction enzymes have been extensively used by geneticists in genetic engineering (see Chapter 19).

CRISPR-Cas Systems

A powerful set of defense mechanisms in bacteria and archaea are **CRISPR-Cas systems**. These have been compared to the immune systems of vertebrates in that they recognize and "remember" DNA from specific pathogens. CRISPR-Cas systems combine small RNA molecules known as CRISPR RNAs (crRNAs) with Cas (CRISPR-associated) proteins to provide defense against specific foreign DNA molecules originating from viruses, plasmids, and transposable elements. CRISPR-Cas systems are widespread in prokaryotes, occurring in 50% of bacterial species and 87% of archaea.

Bacteria and archaea have evolved a diverse set of CRISPR-Cas systems; these are divided into two major classes, 6 types, and 19

subtypes. These different CRISPR-Cas systems vary in the details of their components and mechanisms. In spite of these differences, all CRISPR-Cas systems have two major components: (1) a DNA sequence called the CRISPR array and (2) a set of genes encoding Cas proteins.

CRISPR ARRAY

The **CRISPR array** (**Figure 9.17a**) is located in the bacterial or archaeal chromosome and consists of a series of repeated DNA sequences called clustered regularly interspaced short palindromic repeats (CRISPR). Palindromic sequences are sequences that read the same forward and backward on two complementary DNA strands. A CRISPR array consists of a series of repeated palindromic sequences, separated by spacers that are derived from the DNA of viruses or plasmid genomes. For example, in the bacterium *Pseudomonas aeruginosa*, CRISPR palindromic repeats are 28 base pairs (bp) in length, separated by 32 bp spacers. Adjacent to the CRISPR array is a series of genes that encode **Cas proteins**, which are essential to the functioning of the CRISPR-Cas system.

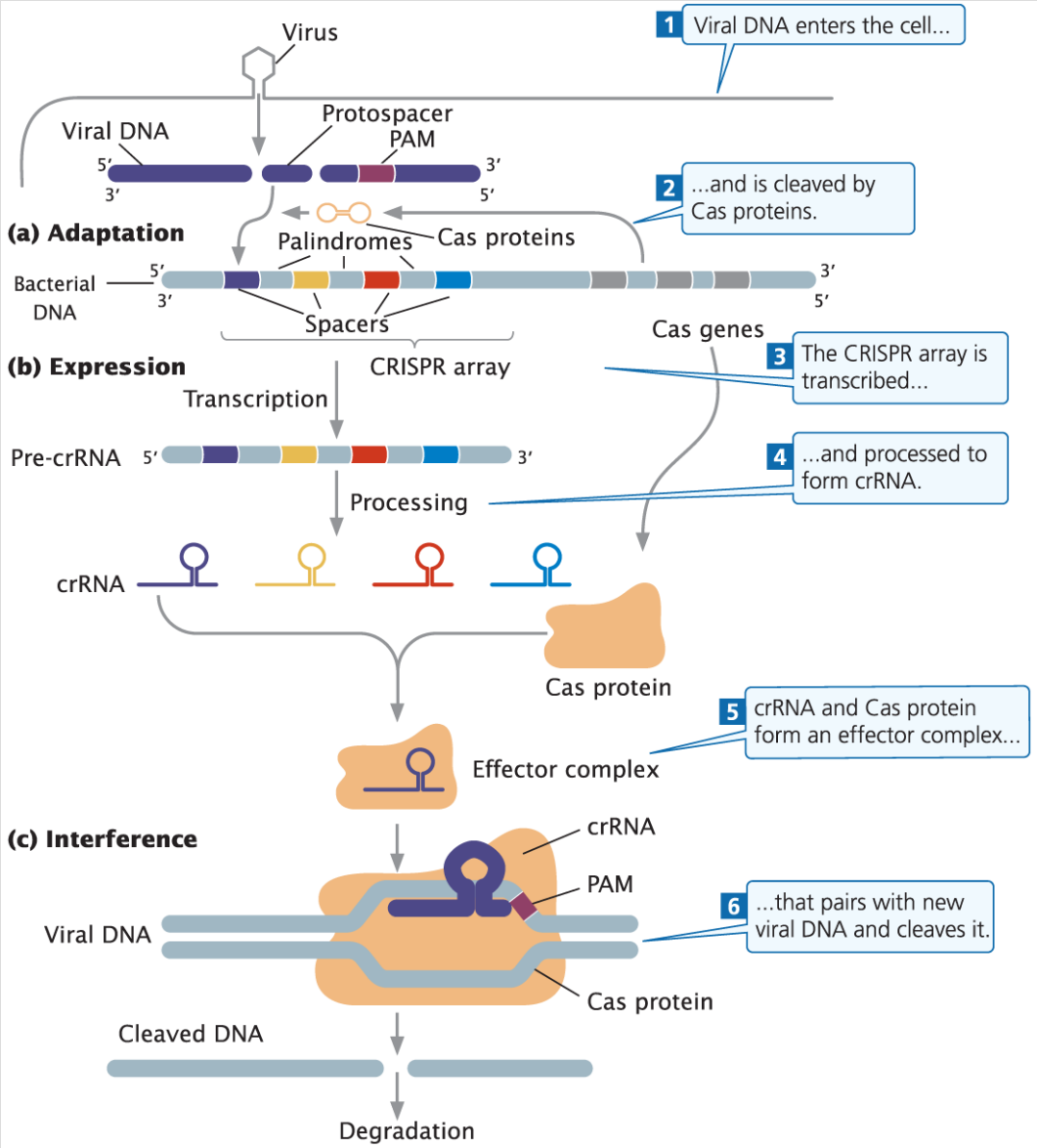
CRISPR-CAS FUNCTION

The action of a CRISPR-Cas system takes place in three stages: adaptation, expression, and interference. Adaptation (Figure 9.17a) occurs when foreign DNA from a virus, plasmid, or other element

enters the cells. The foreign DNA is identified and cleaved by Cas proteins into fragments called protospacers. The protospacers are then processed and incorporated into the CRISPR array as spacers, which serve as a memory of the foreign DNA and can trigger the CRISPR-Cas reaction if the foreign DNA ever enters the cell again. New spacers are preferentially integrated at the 5′ end of the CRISPR array, where they enhance immunity against recent invaders.

In the second stage—expression—the CRISPR array is transcribed into a long CRISPR precursor RNA (**Figure 9.17b**), which is then cleaved by Cas proteins and processed into crRNAs, each of which contains a spacer sequence that is homologous to the foreign DNA. The crRNA combines with a Cas protein to form an effector complex.

Interference, the final stage of the process, occurs when foreign DNA from the same bacteriophage or plasmid enters the cell again. The effector complex binds to the foreign DNA through base pairing between the crRNA and the foreign DNA sequence. The Cas protein cleaves the foreign DNA, which is then degraded (**Figure 9.17c**). In this way, CRISPR-Cas serves as an adaptive defense system against foreign invaders. Because the bacterial cell carries the inserted pieces of foreign DNA in its own chromosome, it can "remember" previously encountered pathogens and quickly destroy their DNA. When bacterial cells divide, they pass on their CRISPR array to progeny cells, so the acquired immunity becomes inherited by future generations of bacteria.



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9.17 CRISPR-Cas immunity takes place in three steps: adaptation, expression, and interference.

Description

The process chart starts with the entry of foreign D N A from a virus into the bacterial cell. Viral D N A consists of complementary strands from 5 prime to 3 prime and from 3 prime to 5 prime respectively, protospacer, and P A M. An arrow points to (a) adaptation where foreign viral D N A is incorporated into the C R I S P R array of bacterial D N A as spacers. Palindromes are embedded between these spacers and Cas proteins. An arrow from the

Cas proteins points to the arrow from the virus to the bacterial D N A. An arrow from the C R I S P R array of adaptation points to (b) expression, where C R I S P R array transcription takes place forming a 5 prime to 3 prime single stranded pre-c r R N A that undergoes processing resulting in the formation of 4 sickle shaped c r R N A. This c r R N A is then incorporated into Cas protein and forms effector complex. After that the process (c) interference takes place where the effector complex binds to the foreign D N A through base pairing between the c r R N A and the foreign D N A sequence along with PAM after that Cas protein cleaves the foreign D N A resulting in degradation of the D N A.

PAM SEQUENCES

Integral to the functioning of most CRISPR-Cas systems is a requirement that foreign DNA that makes up the protospacers possess a short adjacent DNA sequence called a **protospacer-adjacent motif (PAM)**. During interference, the CRISPR-Cas effector complex first associates with a PAM and then unwinds the DNA nearby. The requirement of a PAM for interference solves a dilemma that bacteria face in the use of CRISPR-Cas: how to recognize and cleave foreign DNA without destroying the spacer DNA within its own CRISPR array. To solve this dilemma, many bacteria species have evolved the requirement of an adjacent PAM. Although a PAM is adjacent to the protospacer in the foreign DNA, the PAM sequences themselves are not integrated into the CRISPR array as part of the spacer. In this way, the CRISPR-Cas system cleaves foreign DNA (which has a PAM) but avoids cleaving DNA in the CRISPR array of the bacterial genome (where PAM is absent).

In recent years, researchers have adapted and modified natural CRISPR-Cas systems to serve as powerful gene-editing tools in genetic engineering. We will discuss this use of CRISPR-Cas in Chapter 19.

An Evolutionary Arms Race Between Bacteria and Viruses

As we have seen, bacteria have evolved a number of mechanisms to avoid infection by and reproduction of viruses, but viruses have, in turn, evolved countermeasures to avoid bacterial defenses. This has generated an evolutionary "arms race," in which bacteria and phages are constantly changing in response to evolutionary changes in each other.

One way that viruses can avoid CRISPR-Cas immunity in bacteria is by constantly mutating their sequences so that the bacteria cannot recognize viruses that have previously infected the cell. Viral mutations that are effective in avoiding detection by CRISPR-Cas often occur in the PAM sequence or in the part of the protospacer recognized by the CRISPR-Cas effector complex. In addition, some viruses have evolved small proteins called anti-CRISPRs, which directly interfere with the CRISPR-Cas machinery.

CRISPR-Cas systems are found in many bacteria, but they are missing from almost 50% of all bacterial species. Why do many

bacterial species lack what appears to be a very effective defense against viruses and other foreign DNA? One possibility is that other defense mechanisms are sufficiently effective that CRISPR-Cas immunity provides no additional advantage. There are also likely to be significant costs to having CRISPR-Cas immunity. It may limit the ability of bacteria to take up potentially beneficial DNA that might provide antibiotic resistance or other adaptive traits. It is possible that CRISPR-Cas may sometimes erroneously attack the bacteria's own DNA, creating a type of autoimmunity that disrupts normal function. And there are energetic costs to maintaining the CRISPR-Cas system. All of these factors may make it too costly for some bacteria to evolve CRISPR-Cas immunity.

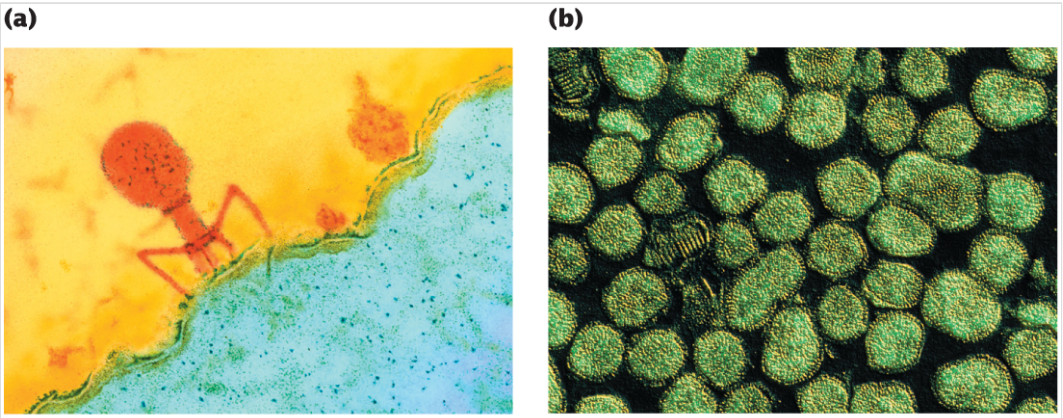
THINK-PAIR-SHARE Question 4

CONCEPTS

Bacteria and archaea have evolved mechanisms to prevent the entry and reproduction of viruses and other forms of invading DNA. These include systems to prevent viral attachment and injection of the viral DNA or RNA into the cell, restriction-modification systems, and CRISPR-Cas immunity. CRISPR-Cas systems are powerful defense mechanisms that provide adaptive immunity: the ability to recognize and remember DNA from specific pathogens. In response, viruses have evolved a number of adaptations to avoid bacterial defenses.

9.5 Viruses Are Simple Replicating Systems Amenable to Genetic Analysis

All organisms—plants, animals, fungi, and bacteria—are infected by viruses. A **virus** is a simple replicating structure made up of nucleic acid surrounded by a protein coat (see Figure 2.4). Viruses come in a great variety of shapes and sizes (**Figure 9.18**). Enveloped viruses have an outer lipid envelope that is derived from the host's cell membrane. Some viruses have DNA as their genetic material, whereas others have RNA; the nucleic acid may be double stranded or single stranded, linear or circular.

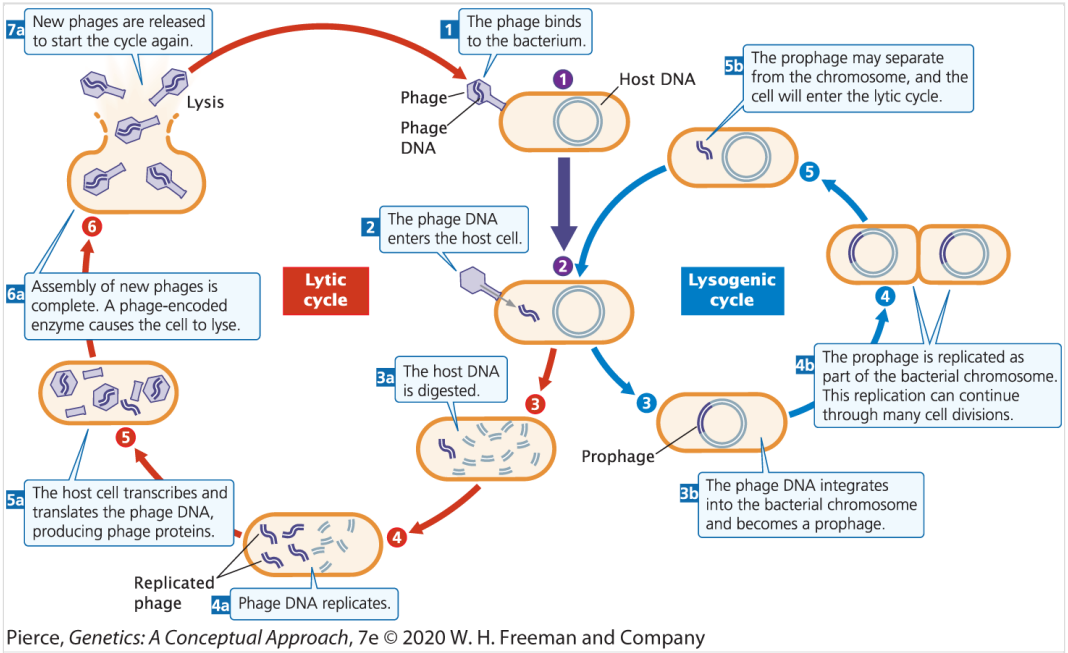


Part a: Biozentrum, University of Basel/Science Source. Part b: Eye of Science/Science Source.

9.18 Viruses have a variety of structures and sizes. (a) Bacteriophage T4 (bright orange). (b) Influenza A virus (green structures).

Viruses that infect bacteria (bacteriophages, or phages for short) have played a central role in genetic research since the late 1940s.

They are ideal for many types of genetic research because they have small and easily manageable genomes, reproduce rapidly, and produce large numbers of progeny. Bacteriophages have two alternative life cycles: the *lytic* cycle and the *lysogenic* cycle. In the lytic cycle, a phage attaches to a receptor on the bacterial cell wall and injects its DNA into the cell (Figure 9.19). Inside the host cell, the host DNA is degraded, and phage DNA is replicated, transcribed, and translated, producing more phage DNA and phage proteins. New phage particles are assembled from these components. The phages then produce an enzyme that breaks open the host cell, releasing the new phages. **Virulent phages** reproduce strictly through the lytic cycle and always kill their host cells.



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9.19 Bacteriophages have two alternative life cycles: lytic and lysogenic.

Description

On the right is the lysogenic cycle, and on the left is the lytic cycle. The host cell has a gray circular chromosome and the phage has a purple double stranded chromosome. The cycles are connected in the middle because they share stages 1 and 2, where the phage binds and enters the host cell. The lytic cycle starts when a phage having phage D N A attaches to bacterial cell containing host D N A. The phage D N A enters the cell. The captions read, 1, The phage binds to the bacterium. 2, The phage D N A enters the host cell. From this point, the phage can either remain in the lytic cycle or can enter lysogenic cycle. In the lytic cycle on the left side, the host D N A is lysed and a caption reads, The host D N A is digested. The next picture shows multiple phage D N A and host D N A fragments and a caption reads, Phage D N A replicates. The next picture of bacterial cell shows phage D N A with phage proteins and then these are assembled and the bacterial cell is lysed. The captions read, Assembly of new phages is complete. A phage-encoded enzyme causes the cell to lyse. New phages are released to start the cycle again. From step 2 in the lytic cycle where phage D N A enters the host cell, the phage can enter a lysogenic cycle. A picture of host cell shows a bacterial chromosome with phage D N A incorporated. A caption reads, The phage D N A integrates into the bacterial chromosome and becomes a prophage. The next picture shows two bacterial cells with phage D N A incorporated into their genome. A caption reads, The prophage is replicated as part of the bacterial chromosome. This replication can continue through many cell divisions. The next picture shows host cell with bacterial chromosome and a separate phage D N A. A caption reads, The prophage may separate and the cell will enter the lytic cycle.

Temperate phages can undergo either the lytic or the lysogenic cycle. The lysogenic cycle begins like the lytic cycle (see Figure 9.19), but inside the cell, the phage DNA integrates into the bacterial chromosome, where it remains as an inactive **prophage**. The prophage is replicated along with the bacterial DNA and is passed on when the bacterium divides. While the prophage is integrated into the bacterial chromosome, it inhibits subsequent infection by the same type of phage. Certain stimuli, such as ultraviolet light and some chemicals, can cause the prophage to dissociate from the

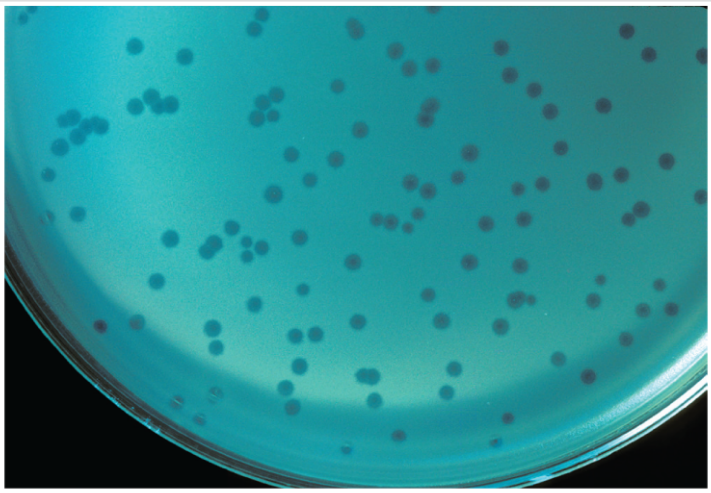
bacterial chromosome and enter the lytic cycle, producing new phage particles and lysing the cell.

THINK-PAIR-SHARE Question 5

Techniques for the Study of Bacteriophages

Viruses reproduce only within host cells, so bacteriophages must be cultured in bacterial cells. Phages can be grown in large liquid cultures of bacteria to generate large numbers of offspring, but to study the characteristics of individual phages, we must isolate them on petri plates. To do so, we can mix phages and bacteria together and plate them on a solid medium. A high concentration of bacteria is used so that the colonies will grow into one another and produce a continuous layer of bacteria, or *lawn*, on the agar. As the bacteria grow, an individual phage infects a single bacterial cell and goes through its lytic cycle. Many new phages are released from the lysed cell and infect additional cells, and the cycle is repeated. Because the bacteria are growing on a solid medium, the diffusion of the phages is restricted, so only nearby cells are infected. After several rounds of phage reproduction, a clear patch of lysed cells, called a **plaque**, appears on the plate (Figure 9.20). Each plaque represents a single phage that multiplied and lysed many cells. Plating of a known volume of a dilute solution of phages on a bacterial lawn and

counting the number of plaques that appear is a way to determine the original concentration of phages in the solution.



Carolina Biological/Medical Images.

9.20 Plaques are clear patches of lysed cells on a lawn of bacteria.

CONCEPTS

Viral genomes may be DNA or RNA, circular or linear, and double or single stranded. Bacteriophages are used in many types of genetic research.

✔ CONCEPT CHECK 6

In which bacteriophage life cycle does the phage DNA become incorporated into the bacterial chromosome?

a. Lytic
b. Lysogenic
c. Both lytic and lysogenic
d. Neither lytic nor lysogenic

Transduction

In our discussion of bacterial genetics in Section 9.3, three mechanisms of gene transfer were identified: conjugation, transformation, and transduction (see Figure 9.7). Let's take a closer look at transduction, in which genes are transferred between bacteria by viruses. In **generalized transduction**, any gene may be transferred. In **specialized transduction**, only a few genes are transferred.

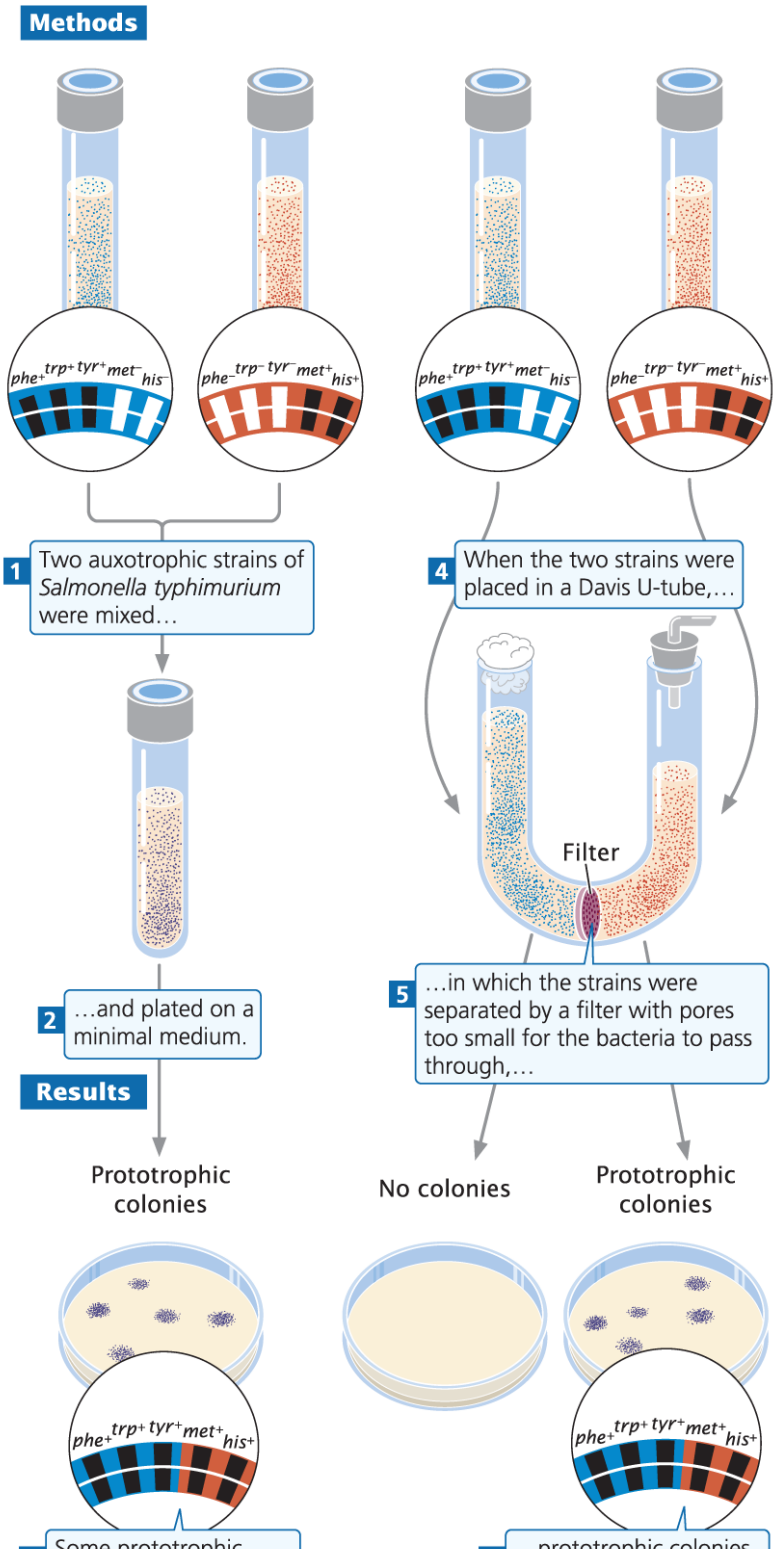
GENERALIZED TRANSDUCTION

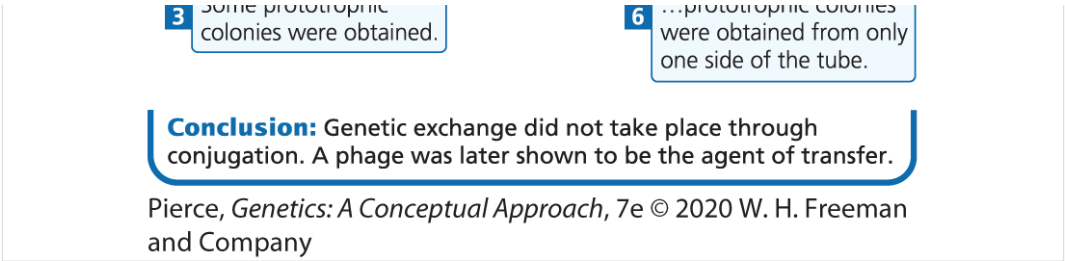
Joshua Lederberg and Norton Zinder discovered generalized transduction in 1952 while trying to produce recombination in the bacterium *Salmonella typhimurium* by conjugation. They mixed a strain of *S. typhimurium* that was phe^+ trp^+ tyr^+ met^- his^- with a strain that was phe^- trp^- tyr^- met^+ his^+ (**Figure 9.21**) and plated the mixture on a minimal medium. A few prototrophic recombinants (phe^+ trp^+ tyr^+ met^+ his^+) appeared, suggesting that conjugation had taken place. However, when they tested the two strains in a U-shaped tube similar to the one used by Davis, some phe^+ trp^+ tyr^+ met^+ his^+ prototrophs were obtained on one side of the tube (compare Figure 9.21 with Figure 9.9). In this apparatus, the two strains were separated by a filter with pores too small for the passage of bacteria, so how were genes being transferred between bacteria in the

absence of conjugation? The results of subsequent studies revealed that the agent of transfer was a bacteriophage.

Experiment

Question: Does genetic exchange between bacteria always require cell-to-cell contact?





9.21 The Lederberg and Zinder experiment.

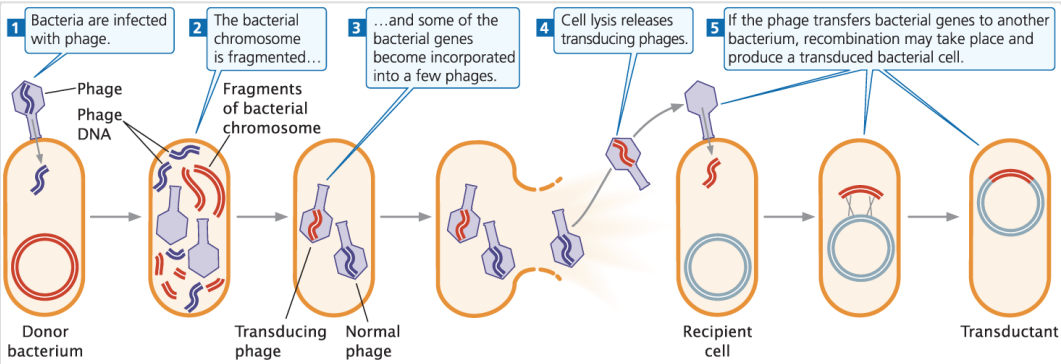
Description

The experiment starts with a question, Does genetic exchange between bacteria always require cell-to-cell contact? The methods below show two auxotrophic strains of Salmonella typhimurium in two separate test tubes and a zoomed in image of their bacterial chromosome. A caption reads, Two auxotrophic strains of Salmonella typhimurium were mixed and plated on minimal medium. Next test tube shows mixed strains. The results section below shows a petriplate with colonies labeled prototrophic colonies and a zoomed in image of their chromosome. A caption reads, Some prototrophic colonies were obtained. In another method, the two strains are not mixed. A caption reads, When the 2 strains were placed in a Davis U-tube, in which the strains were separated by a filter with pores too small for the bacteria to pass through, prototrophic colonies were obtained from only one side of the tube. The two strains are put into a U-shaped tube that has a filter in between physically separating the two bacteria. Two petriplates are shown below in the results section. The first has no colonies and the second has some colonies labeled prototrophic colonies. A caption below reads, Conclusion: Genetic exchange did not take place through

conjugation. A phage was later shown to be the agent of transfer.

In the lytic cycle of phage reproduction, the phage degrades the bacterial chromosome into random fragments (Figure 9.22). In some types of bacteriophages, a piece of the bacterial chromosome, instead of phage DNA, occasionally gets packaged into a phage coat; these phage particles are called **transducing phages**. If a transducing phage infects a new cell and releases the bacterial DNA, the

introduced genes may then become integrated into the bacterial chromosome by a double crossover. Some transducing phages insert viral DNA, along with the bacterial DNA, into the bacterial chromosome. In either case, bacterial genes can be moved from one bacterial strain to another, producing recombinant bacteria called **transductants**.



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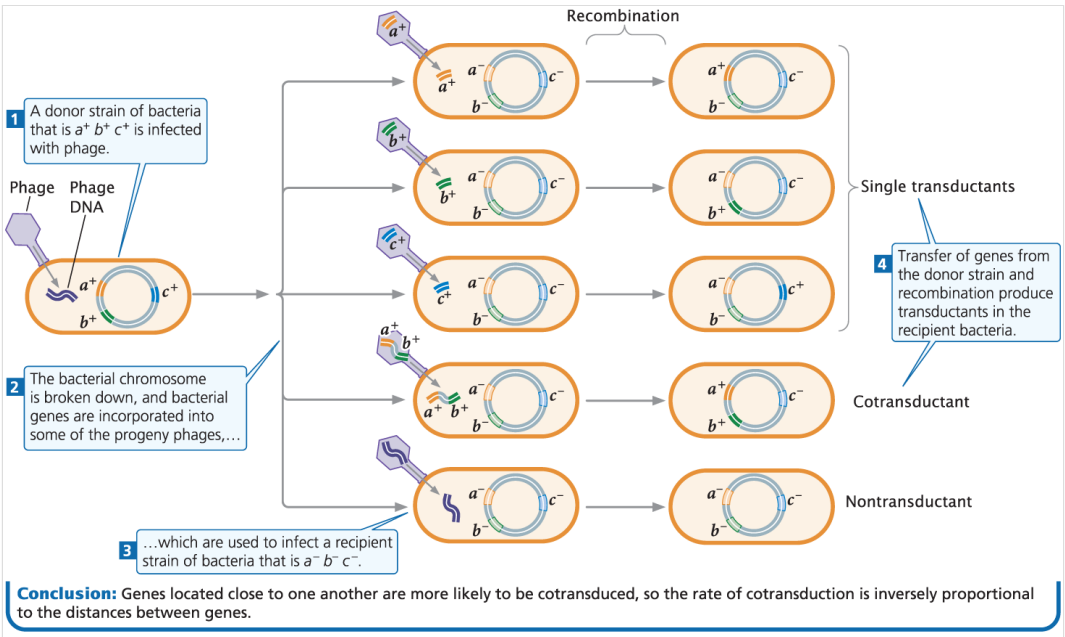
9.22 Genes can be transferred from one bacterium to another through generalized transduction.

Description

The illustration shows a bacterium with a phage infecting it at the left. The bacterial chromosome is red, circular and the phage D N A is purple double stranded. A caption reads, Bacteria are infected with phage. This leads to the next image that shows the bacterial cell with D N A fragments of both the phage and the bacterium and new phages with no D N A. A caption reads, The bacterial chromosome is fragmented and some of the bacterial genes become incorporated into a few phages. This leads to the next image that shows assembled phages inside the host cell. One is transducing phage having bacterial chromosome and the other is normal phage having its own D N A. Bacterial cell is then lysed and the phage particles are released. A caption reads, cell lysis releases transducing phages. The transducing phage infects another host cell and its D N A enters the bacterial cell. Crossing over occurs and a caption reads If the phage transfers bacterial genes to another bacterium, recombination may take place and produce a transduced bacterial cell. This leads to the next image that shows transductant with recombinant D N A.

Not all phages are capable of transduction, a rare event that requires that (1) the phage degrade the bacterial chromosome, (2) the process of packaging DNA into the phage coat not be specific for phage DNA, and (3) the bacterial genes transferred by the virus recombine with the chromosome in the recipient cell. The overall rate of transduction ranges from only about 1 in 100,000 to 1 in 1,000,000 phage particles.

Because of the limited size of a phage particle, only about 1% of the bacterial chromosome can be transduced. Only genes located close together on the bacterial chromosome will be transferred together, or **cotransduced**. Because the chance of a cell undergoing transduction by two separate phages is exceedingly small, we can assume that any cotransduced genes are located close together on the bacterial chromosome. Thus, rates of cotransduction, like rates of cotransformation, provide an indication of the physical distances between genes on a bacterial chromosome, and transduction has been used in the past to map genes in bacteria (**Figure 9.23**). **› TRY PROBLEM 29**



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9.23 Generalized transduction can be used to map genes.

Description

The illustration shows a phage with purple D N A and a bacterial chromosome with genes a superscript positive, b superscript positive, and c superscript positive. A caption reads, A donor strain of bacteria that is a superscript positive b superscript positive c superscript positive is infected with phage. The second caption reads, The bacterial chromosome is broken down, and bacterial genes are incorporated into some of the progeny phages, which are used to infect a recipient strains of bacteria that is a superscript negative b superscript negative c superscript negative. 5 forward arrows show 5 different phage infecting 5 different bacterial cells. One has gene, a, packaged; one has gene, b; one has gene, c; one has genes a and b; and one has phage D N A. Recombination occurs and the resulting bacterial chromosomes are as follows: 1, bacterium with gene a superscript positive, b superscript negative, and c superscript negative; 2, bacterium with gene a superscript negative, b superscript positive, and c superscript negative; 3, bacterium with gene a superscript negative, b superscript negative, and c superscript positive; 4, bacterium with gene a superscript positive, b superscript positive, and c superscript negative; 5, bacterium with gene a superscript negative, b superscript negative, and c superscript negative. The first to third bacterium are labeled single transductants. The fourth is labeled co-transductant and fifth is labeled non-tranductant. A caption reads,

Transfer of genes from the donor strain and recombination produce transductants in the recipient bacteria.

CONCEPTS

In transduction, bacterial genes are packaged into a phage coat, transferred to another bacterium by the virus, and incorporated into the recipient bacterial chromosome by crossing over. Bacterial genes can be mapped with the use of generalized transduction.

✔ CONCEPT CHECK 7

In gene mapping using generalized transduction, bacterial genes that are cotransduced are

a. far apart on the bacterial chromosome.
b. on different bacterial chromosomes.
c. close together on the bacterial chromosome.
d. on a plasmid.

SPECIALIZED TRANSDUCTION

Like generalized transduction, specialized transduction involves gene transfer from one bacterium to another by a phage, but here, only genes near particular sites on the bacterial chromosome are transferred. This process requires temperate bacteriophages that use specific prophage integration sites on the bacterial chromosome. When the lysogenic cycle ends, the prophage may be imperfectly excised from the bacterial chromosome, carrying with it a small part of the bacterial DNA adjacent to the integration site. A

phage carrying this DNA then injects it into another bacterial cell in the next round of infection. This process resembles conjugation in F′ cells, in which the F plasmid carries genes from one bacterium into another (see Figure 9.14). Specialized transduction requires phages that use specific integration sites; many phages integrate randomly and exhibit only generalized transduction.

CONCEPTS

Specialized transduction transfers only those bacterial genes located near the site of prophage integration.

Gene Mapping in Phages

The mapping of genes in bacteriophages depends on homologous recombination between phage chromosomes and therefore requires the application of the same principles that are applied to mapping genes in eukaryotic organisms (see Chapter 7). Crosses are made between viruses that differ in two or more genes, and recombinant progeny phages are identified and counted. The proportion of recombinant progeny is then used to estimate the distances between the genes and to determine their linear order on the chromosome.

In 1949, Alfred Hershey and Raquel Rotman examined rates of recombination in the bacteriophage T2, which has single-stranded

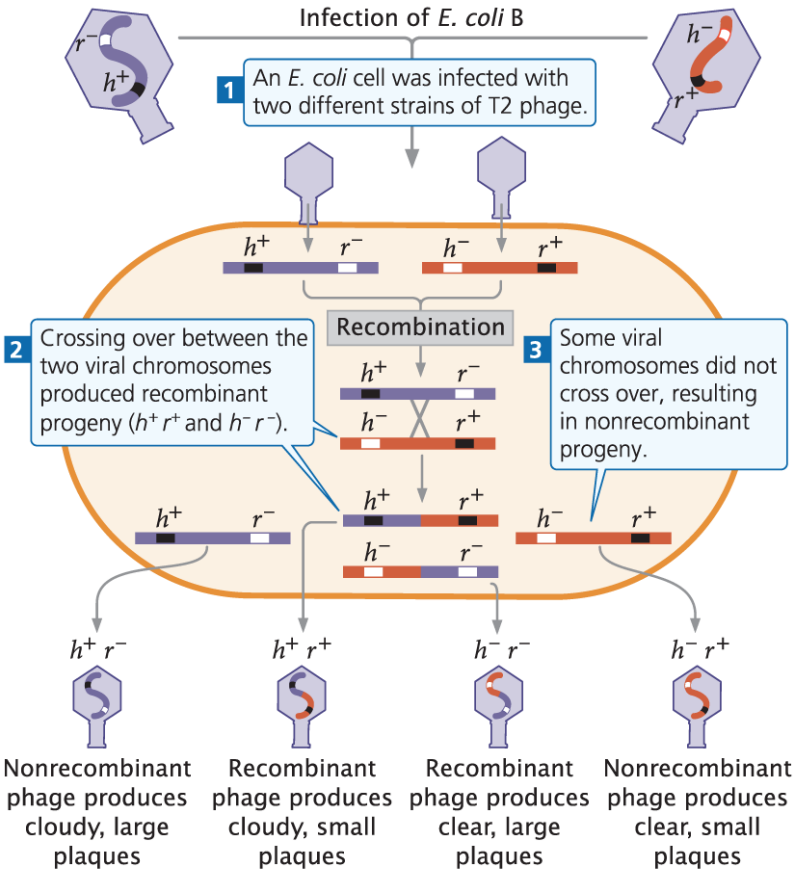
DNA. They studied recombination between genes in two strains that differed in plaque appearance and host range (the bacterial strains that the phages could infect). One strain was able to infect and lyse type B *E. coli* cells but not type B/2 cells (making this strain of phage wild type with a normal host range, or h^+), and produced abnormal plaques that were large with distinct borders (r^-). The other strain was able to infect and lyse both B and B/2 cells (mutant host range, h^-) and produced wild-type plaques that were small with fuzzy borders (r^+).

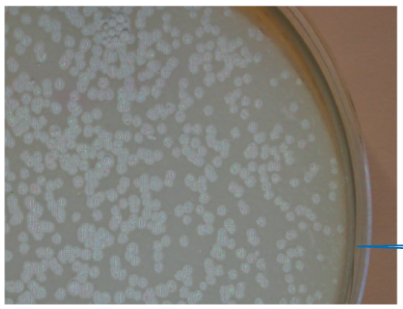
Hershey and Rotman crossed the $h^+ r^-$ and $h^- r^+$ strains of T2 by infecting type B *E. coli* cells with a mixture of the two strains. They used a high concentration of phages so that most cells could be simultaneously infected by both strains (**Figure 9.24**). Within the bacterial cells, homologous recombination occasionally took place between the chromosomes of the different bacteriophage strains, producing $h^+ r^+$ and $h^- r^-$ chromosomes, which were then packaged into new phage particles. When the cells lysed, the recombinant phages were released, along with nonrecombinant $h^+ r^-$ phages and $h^- r^+$ phages.

Experiment

Question: How can we determine the position of a gene on a phage chromosome?

Method





4 Progeny phages were then plated on a mixture of *E. coli* B and *E. coli* B/2 cells,...

Results

Genotype	Plaques	Designation
$h^- r^+$	42	Parental progeny 76%
$h^+ r^-$	34	
$h^+ r^+$	12	Recombinant progeny 24%
$h^- r^-$	12	

5 ...which allowed all four genotypes of progeny to be identified.

6 The percentage of recombinant progeny allowed the h^- and r^- mutations to be mapped.

recombinant plaques $(h^+ r^+) + (h^- r^-)$

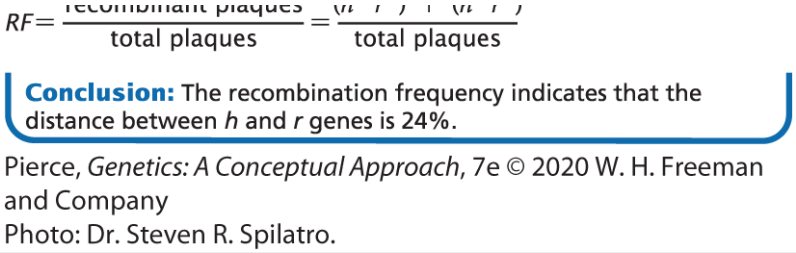


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Photo: Dr. Steven R. Spilatro.

9.24 Hershey and Rotman developed a technique for mapping viral genes.

Description

The illustration starts with a question that reads, How can we determine the position of a gene on a phage chromosome? The illustration below has a methods and a results section. In the methods section, there is a picture of a phage with purple linear D N A and a phage with red linear D N A. One of them is r superscript negative and h superscript positive, and the other phage has the opposite genotype, r superscript positive h superscript negative. A caption reads, An E coli cell was infected with 2 different strains of T 2 phage. Both the phages infect a bacterial cell and inject their D N A into the same cell. The phage D N A recombines inside the host cell. Two captions read as follows: Crossing over between the 2 viral chromosomes produced recombinant progeny, h superscript positive r superscript positive and h superscript negative r superscript negative and Some viral chromosomes did not cross over, resulting in non-recombinant progeny. Arrows beneath the bacterial cell show 4 different combinations of genes assembled into phage genomes followed by a text as follows: 1, h superscript positive r superscript negative, non-recombinant phage produces cloudy, large plaques; 2, h superscript positive r superscript positive, recombinant phage produces cloudy, small plaques; 3, h superscript negative r superscript negative, recombinant phage produces clear, large plaques; and 4, h superscript negative r superscript positive, non-recombinant phage produces clear, small plaques. Below this, an image of bacteria in a petri plate with different types of plaques. A caption reads, Progeny phages were then plated on a mixture of E coli B and E coli B over 2 cells, which allowed all four genotypes of progeny to be identified. In the results section below, is a table showing the number of plaques of each phage genotype and designation. The table has 3 columns and 4 rows. The column headers are as follows: Genotype, plaques, and designation. For genotype h superscript negative r superscript positive, there are 42 plaques. For genotype h superscript positive r superscript negative, there are 34 plaques. Designation for both is parental progeny 76 percent. For genotype h superscript positive r superscript positive, there are 12 plaques. For genotype h superscript negative r

superscript negative, there are 12 plaques. Designation for both is recombinant progeny 24 percent. A formula for calculating the R F, is given below: R F equals start fraction recombinant plaques over total plaques end fraction equals start fraction left parenthesis h superscript positive r superscript positive right parenthesis plus left parenthesis h superscript negative r superscript negative right parenthesis over total plaques end fraction. A caption reads, The percentage of recombinant progeny allowed the h superscript negative and r superscript negative mutants to be mapped. A caption reads, Conclusion: The recombination frequency indicates that the distance between h and r genes is 24 percent.

Hershey and Rotman diluted the progeny phages and plated them on a bacterial lawn that consisted of a *mixture* of B and B/2 cells. Phages carrying the h^+ allele (which conferred the ability to infect only B cells) produced a cloudy plaque because the B/2 cells were not lysed. Phages carrying the h^- allele produced a clear plaque because all the cells within the plaque were lysed. The r^+ phages produced small plaques, whereas the r^- phages produced large plaques. The genotypes of the progeny phages could therefore be determined by the appearance of the plaques (see Figure 9.24 and **Table 9.4**).

TABLE 9.4 Progeny phages produced from $h^- r^+ \times h^+ r^-$

Phenotype	Genotype
Clear and small	$h^- r^+$
Cloudy and large	$h^+ r^-$
Cloudy and small	$h^+ r^+$
Clear and large	$h^- r^-$

In this type of phage cross, the recombination frequency (*RF*) between the two genes can be calculated by using the following formula:

$$RF = \frac{\text{recombinant plaques}}{\text{total plaques}}$$

In Hershey and Rotman's cross, the recombinant plaques were $h^+ r^+$ and $h^- r^-$, so the recombination frequency was

$$RF = \frac{(h^+r^+) + (h^-r^-)}{\text{total plaques}}$$

Recombination frequencies can be used to determine the distances between genes and their order on the phage chromosome, just as they are used to map genes in eukaryotes. Today, most phage genes are mapped by DNA sequencing. **› TRY PROBLEMS 28 AND 32**

CONCEPTS

To map phage genes, bacterial cells are infected with viruses that differ in two or more traits. Recombinant plaques are counted, and rates of recombination are used to determine the linear order of the genes on the chromosome and the distances between them.

Plant and Animal Viruses

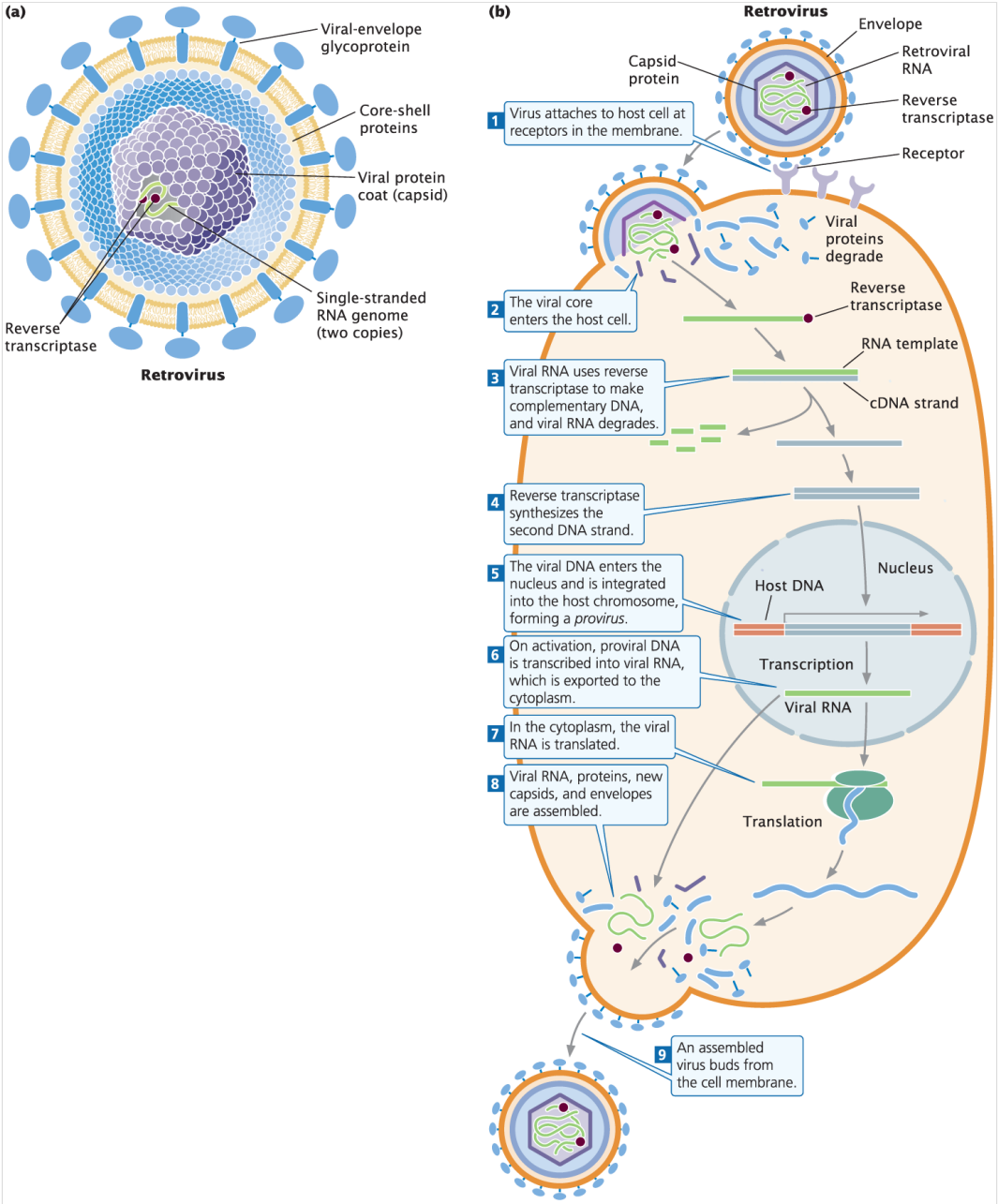
Thus far, we have primarily considered viruses that infect bacteria. Viruses also infect protists, fungi, plants, humans, and other animals, and some are important pathogens in these organisms. About 263 different viruses are known to infect humans, although there are undoubtedly many that have not been characterized. What we have learned about bacteriophages has important implications for the study of viruses that infect these complex organisms.

Viral genomes may be encoded in either DNA or RNA and may be double stranded or single stranded. Double-stranded DNA viruses include adenoviruses, which are responsible for gastroenteritis and some types of pneumonia; herpesviruses, which cause genital herpes and cold sores; papillomaviruses, which are associated with some cases of cervical cancer (see Chapter 23); and numerous viruses that infect other vertebrate and invertebrate animals. Single-stranded DNA viruses include parvovirus (parvo), which is highly infectious and sometimes lethal in dogs.

RNA viruses are found in a wide range of host organisms, including plants, invertebrates, and vertebrates. Almost all viruses that infect both plants and all major groups of vertebrates have RNA genomes. RNA is also the genetic material of some human viruses, including those that cause colds, influenza, polio, and AIDS. The medical and economic importance of RNA viruses has encouraged their study.

RNA viruses capable of integrating into the genomes of their hosts, much as temperate phages insert themselves into bacterial

chromosomes, are called **retroviruses** (**Figure 9.25a**). Because the retroviral genome is RNA, whereas that of the host is DNA, a retrovirus must produce **reverse transcriptase**, an enzyme that can synthesize complementary DNA (cDNA) from either an RNA or a DNA template. A retrovirus uses reverse transcriptase to copy its RNA genome into a single-stranded DNA molecule. The reverse transcriptase enzyme—or sometimes the host DNA polymerase—then copies this single-stranded DNA, creating a double-stranded DNA molecule that is integrated into a host chromosome. A viral genome incorporated into a host chromosome is called a **provirus**. The provirus is replicated by host enzymes when the host chromosome is duplicated (**Figure 9.25b**).



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9.25 A retrovirus uses reverse transcription to incorporate its RNA into the host DNA. (a) Structure of a typical retrovirus. Two copies of the single-stranded RNA genome and the reverse transcriptase enzyme are shown enclosed within a protein capsid. The capsid is surrounded by a viral envelope that is studded with viral glycoproteins. (b) The retrovirus life cycle.

Description

Illustration a on the left shows the typical structure of a retrovirus. Two copies of single-stranded R N A molecule is present with reverse transcriptase on it. R N A is enclosed within an octahedral viral protein coat, also known as capsid. Circular core-shell proteins are present above it, enclosing the whole structure. Viral-envelope glycoprotein lie on top of that in a radial manner.

Illustration b on the right shows how a retrovirus uses reverse transcription to incorporate its R N A into the host D N A. The illustration starts with a retrovirus docking on a cell receptor of a bacterial cell and then fusing with the cell. The first caption reads, Virus attaches to host cell at receptors in the membrane. Viral R N A with reverse transcriptase enzyme and viral proteins enters the host cell. The proteins are labeled viral proteins degrade. The second caption reads, The viral core enters the host cell. Reverse transcriptase on R N A makes c D N A strand complementary to the R N A. The third caption reads, Viral R N A uses reverse transcriptase to make complementary D N A, and viral R N A degrades. From this c D N A, a second complementary strand is formed. The fourth caption reads, Reverse transcriptase synthesizes the second D N A strand. This D N A now enters the host nucleus and incorporates itself within the host D N A. Transcription occurs and viral R N A is produced. This R N A leaves the nucleus and produces viral protein in the cell through translation. Assembly of virus takes place and a new virus is produced. The next captions read as follows: The viral D N A enters the nucleus and is integrated into the host chromosome, forming a provirus. On activation, proviral D N A is transcribed into viral R N A, which is exported to the cytoplasm. In the cytoplasm, the viral R N A is translated. Viral R N A, proteins, new capsids, and envelopes are assembled. An assembled virus buds from the cell membrane.

When conditions are appropriate, the provirus undergoes transcription to produce numerous copies of the original viral RNA genome. This RNA encodes viral proteins and serves as genomic RNA for new viral particles. As these new viruses escape the cell, they collect patches of the cell membrane to use as their envelopes.

All known retroviral genomes have three genes in common: *gag*, *pol*, and *env*, each encoding a precursor protein that is cleaved into two

or more functional proteins. The *gag* gene encodes proteins that make up the viral protein coat. The *pol* gene encodes reverse transcriptase and an enzyme called **integrase**, which inserts the viral DNA into a host chromosome. The *env* gene encodes the glycoproteins that appear on the surface of the viral envelope.

Some retroviruses contain **oncogenes** (see Chapter 23), which may stimulate cell division and cause the formation of tumors. The first retrovirus to be isolated, the Rous sarcoma virus, was originally recognized by its ability to produce connective-tissue tumors (sarcomas) in chickens.

THINK-PAIR-SHARE Question 6

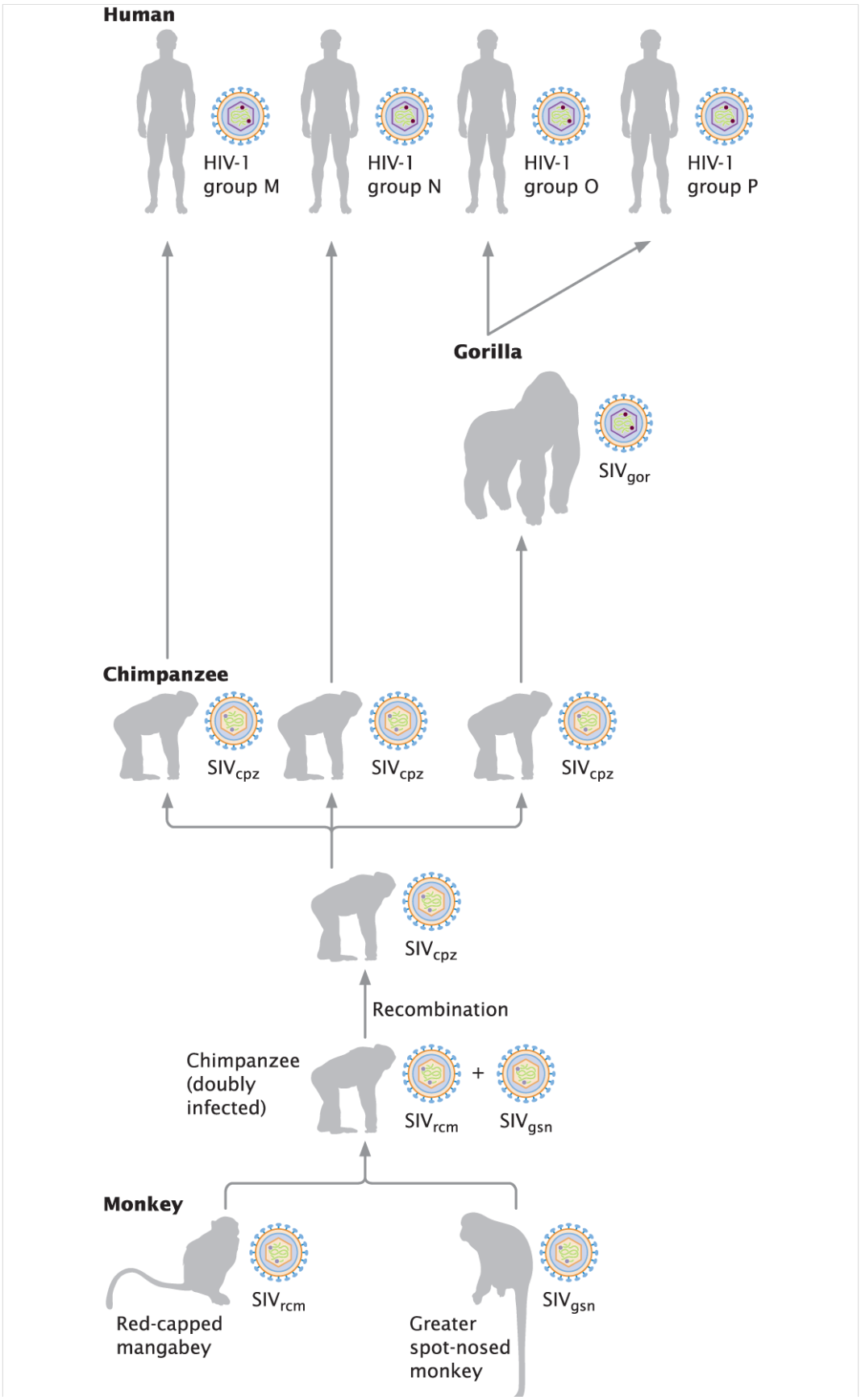
Human Immunodeficiency Virus and AIDS

An example of a retrovirus is the human immunodeficiency virus (HIV), which causes acquired immune deficiency syndrome (AIDS). Although research now indicates that HIV entered the United States around 1970 and was present in Africa much earlier, AIDS was not recognized as a disease until 1982, when a number of homosexual males in the United States began to exhibit symptoms of a new immune-system-deficiency disease. In that year, Robert Gallo proposed that AIDS was caused by a retrovirus; eventually, research

by Gallo, Luc Montagnier, Françoise Barré-Sinoussi, Jay Levy, and others identified HIV as the causative agent of AIDS. Between 1983 and 1984, as the AIDS epidemic became widespread, the HIV retrovirus was isolated from people with the disease. AIDS is now known to be caused by two different immunodeficiency viruses, HIV-1 and HIV-2, which together have infected more than 70 million people worldwide since the beginning of the epidemic. Of those infected, 35 million have died. At the end of 2017, there were almost 37 million people around the globe living with HIV, including 2 million children. Most cases of AIDS are caused by HIV-1, which now has a global distribution; HIV-2 is found primarily in western Africa.

HIV illustrates the importance of genetic recombination in viral evolution. Studies of the DNA sequences of HIV and other retroviruses reveal that HIV-1 is closely related to the simian immunodeficiency virus found in chimpanzees (SIV_{cpz}). Many wild chimpanzees in Africa are infected with SIV_{cpz}, although it doesn't cause AIDS-like symptoms in these animals. SIV_{cpz} is itself a hybrid that resulted from recombination between a retrovirus found in the red-capped mangabey (a monkey) and a retrovirus found in the greater spot-nosed monkey (**Figure 9.26**). Apparently, one or more chimpanzees became infected with both viruses; recombination between the viruses produced SIV_{cpz}, which was then transmitted to humans through contact with infected chimpanzees. In humans, SIV_{cpz} underwent significant evolution to become HIV-1, which then spread throughout the world to produce the AIDS epidemic. Several independent transfers of SIV_{cpz} to humans gave rise to different

groups (strains) of HIV-1. In the case of HIV-1 groups O and P, SIV_{cpz} first jumped to gorillas, then passed from gorillas to humans. The M group of HIV-1 has spread throughout the world and is responsible for most cases of HIV worldwide. The O group has infected about 100,000 people in west central Africa. Groups N and P have infected only a small number of people in Africa. HIV-2 evolved from a different retrovirus, SIV_{sm}, found in sooty mangabeys.


Human
HIV-1 group M
HIV-1 group N
HIV-1 group O
HIV-1 group P
Gorilla
SIVgor
Chimpanzee
SIVcpz
SIVcpz
SIVcpz
SIVcpz
Recombination
Chimpanzee (doubly infected)
SIVrcm
+
SIVgsn
Monkey
SIVrcm
Red-capped mangabey
SIVgsn
Greater spot-nosed monkey

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9.26 HIV-1 evolved from a similar virus SIV_{cpz} found in chimpanzees and was transmitted to humans. SIV_{cpz} arose from recombination between retroviruses from red-capped mangabeys and from greater spot-nosed monkeys.

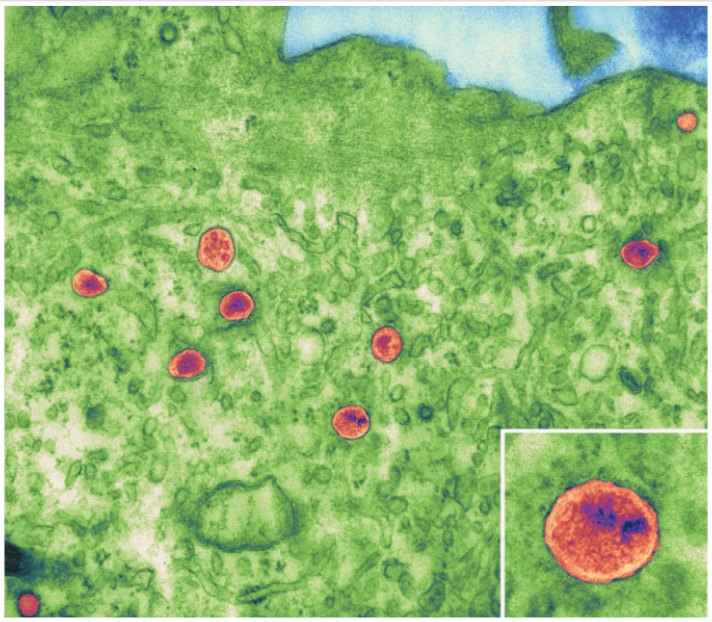
Description

The illustration shows outlines of different human and non-human primates with a drawing of their respective S I V or H I V viruses. At the bottom of the illustration, is the red-capped mangabey wth S I V subscript r c m and greater spot-nosed monkey with S I V subscript g s n. An upward arrow points to a doubly infected chimpanzee with S I V subscript r c m and S I V subscript g s n. Recombination occurs and an upward arrow shows an outline of a chimpanzee with a virus called S I V subscript c p z. From this chimpanzee, three arrows pointing up show three chimpanzees with the same virus, S I V c p z. From the first chimpanzee, an upward arrow points to a human outline with H I V-1 group M. From the second chimpanzee, an upward arrow points to a human outline with H I V-1 group N. An arrow from the third chimpanzee leads to a gorilla outline with S I V g o r. From this gorilla, two upward arrows point to two individual human outlines; one with H I V-1 group O and the other with H I V-1 group P.

HIV is transmitted between humans by sexual contact and through any type of blood-to-blood contact, such as that caused by the sharing of dirty needles by drug users. HIV can also be transmitted between mother and child during pregnancy or in breast milk. Until screening tests were developed to identify HIV-infected blood, transfusions and clotting factors used to treat hemophilia were sources of infection as well.

HIV principally attacks a class of blood cells called helper T lymphocytes, or simply helper T cells (Figure 9.27). HIV enters a

helper T cell, undergoes reverse transcription, and integrates into the chromosome. The virus reproduces rapidly, destroying the T cell as new virus particles escape from the cell. Because helper T cells are central to immune function, people with AIDS have a diminished immune response; most deaths among AIDS patients are caused by secondary infections that develop because they have lost the ability to fight off pathogens.



Thomas Deerinck, NCMIR/Science Source.

9.27 HIV principally attacks helper T lymphocytes. This electron micrograph shows a T cell (green) infected with HIV (orange).

The HIV genome is 9749 nucleotides long and carries *gag*, *pol*, *env*, and six other genes that regulate the life cycle of the virus. HIV's reverse transcriptase is very error prone, giving the virus a high mutation rate and allowing it to evolve rapidly, even within a single host. This rapid evolution makes the development of an effective

vaccine against HIV particularly difficult. Genetic variation within the human population also affects the virus. To date, more than 10 loci in humans that affect HIV infection and the progression of AIDS have been identified.

CONCEPTS

A retrovirus is an RNA virus that integrates into a host chromosome by using reverse transcription to make a DNA copy of its RNA genome. Human immunodeficiency virus, the causative agent of AIDS, is a retrovirus. It evolved from related retroviruses found in other primates.

✔ CONCEPT CHECK 8

What enzyme is used by a retrovirus to make a DNA copy of its genome?

Influenza

Influenza demonstrates how rapidly changes in a pathogen can arise through recombination of its genetic material. Influenza, commonly called flu, is a respiratory disease caused by influenza viruses. In the United States, from 5% to 20% of the population is infected with influenza annually, and though most cases are mild, an estimated 36,000 people die from influenza-related causes each year. At certain times, particularly when new strains of influenza virus enter the human population, there are worldwide epidemics (called

pandemics); for example, in 1918, the Spanish flu virus killed an estimated 20 million to 100 million people worldwide.

Influenza viruses are RNA viruses that infect birds and mammals. The three main types are influenza A, influenza B, and influenza C. Most cases of the common flu are caused by influenza A and B. Influenza A is divided into subtypes on the basis of two proteins, hemagglutinin (HA) and neuraminidase (NA), found on the surface of the virus. The HA and NA proteins affect the ability of the virus to enter host cells and the host organism's immune response to infection. There are 16 types of HA and 9 types of NA, which can exist in a virus in different combinations. For example, common subtypes of influenza A circulating in humans today are H1N1 and H3N2 (Table 9.5), along with several types of influenza B.

TABLE 9.5 Strains of influenza virus responsible for major flu pandemics

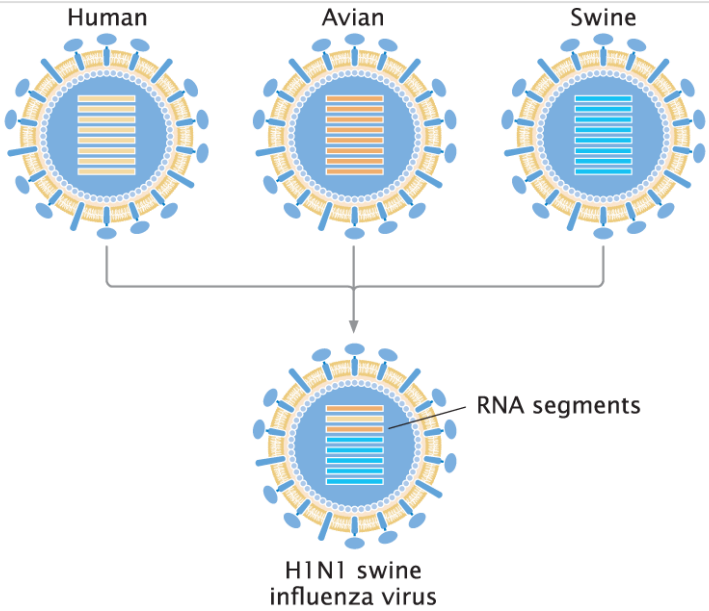
Year	Influenza Pandemic	Strain
1918	Spanish flu	H1N1
1957	Asian flu	H2N2
1968	Hong Kong flu	H3N2
2009	Swine flu	H1N1

Although the influenza virus is an RNA virus, it is not a retrovirus: its genome is not copied into DNA and incorporated into a host chromosome. The influenza viral genome consists of seven or eight pieces of RNA enclosed in a viral envelope. Each piece of RNA

encodes one or two of the virus's proteins. The virus enters a host cell by attaching to specific receptors on the cell membrane. After the viral particle has entered the cell, the viral RNA is released, copied, and translated into viral proteins. Viral RNA molecules and viral proteins are then assembled into new viral particles, which exit the cell and infect additional cells.

One of the dangers of the influenza virus is that it evolves rapidly, so that new strains appear frequently. The virus evolves in two ways. First, the enzyme that copies the viral RNA is especially prone to making mistakes, so new mutations are continually introduced into the genome of each viral strain. This type of continual change is called **antigenic drift**. Second, major changes in the viral genome occasionally take place through **antigenic shift**, in which genetic material from different strains is combined in a process called reassortment. Reassortment may take place when a host is simultaneously infected with two different strains. When the RNAs of both strains are replicated within the cell, RNA segments from two different strains may be incorporated into the same viral particle, creating a new strain. For example, in 2002, reassortment occurred between the H1N1 and H3N2 subtypes, creating a new H1N2 strain that contained the HA from H1N1 and the NA from H3N2. New strains produced by antigenic shift are responsible for most pandemics because no one has immunity to the radically different virus that is produced.

Birds harbor the most different strains of influenza A, but humans are not easily infected with bird influenza. New strains in humans are thought to arise most often from viruses that reassort in pigs, which can be infected by viruses from both humans and birds. In 2009, a new strain of H1N1 influenza (called swine flu) emerged in Mexico and quickly spread throughout the world. This virus arose from a series of reassortment events that combined gene sequences from human, bird, and pig influenza viruses (Figure 9.28). Farming practices that raise pigs and birds in close proximity may facilitate reassortment among avian, swine, and human strains of influenza.



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9.28 New strains of influenza virus are created by reassortment of genetic material from different strains. A new H1N1 virus (swine flu) that appeared in 2009 contained genetic material from avian, swine, and human viruses.

Description

The illustration shows images of viruses with different colored R N A segments inside. The swine virus has 8 blue-colored R N A segments inside. The human strain's segments are yellow, while the avian's segments are orange. In the H 1 N 1 swine influenza virus below, there are 5 blue segments, 2 orange segments, and 1 yellow segment inside the virus.

Rhinoviruses

Rhinoviruses are very small RNA viruses that infect epithelial cells of the respiratory tract and are responsible for respiratory infections, including the common cold. Although the symptoms of rhinovirus infections are usually mild, they constitute a serious public health burden: colds force many people to miss work, make millions of people miserable, and occasionally lead to medical complications, such as pneumonia or exacerbation of asthma and cystic fibrosis. Respiratory infections caused by rhinoviruses remain resistant to treatment, even in the era of modern medicine.

Rhinoviruses have a genome of single-stranded RNA, consisting of approximately 7200 nucleotides, that encodes only 11 proteins. Rhinoviruses have considerable genetic diversity and evolve rapidly. These viruses have a high mutation rate, caused by the inability of their RNA polymerase enzymes (which copy the RNA genome of the virus) to detect and correct mistakes made during the copying process. In addition, multiple viral strains may simultaneously infect the same cell and recombine, leading to the emergence of new rhinovirus strains.

Rhinoviruses that cause colds in humans are highly specialized and are adapted to the human ecosystem. Curiously, they evolved from enteroviruses—viruses that inhabit the gut and can cause acute intestinal infections. Research suggests that rhinoviruses evolved long ago and are adapted to replicate and spread between human cells efficiently without killing their host, leading to relatively mild effects on the humans they infect. Genetic analysis demonstrates that there are three species of human rhinoviruses, called rhinovirus A, rhinovirus B, and rhinovirus C. Within each species, there are numerous variants, called serotypes. Recombination within and between species constantly generates new types of rhinoviruses.

Zika Virus

Zika virus is an RNA flavivirus, a group that includes a number of viruses that cause human disease, including West Nile virus, dengue virus, tick-borne encephalitis virus, and yellow fever virus. Zika virus has recently spread rapidly around the world and is transmitted to humans by *Aedes* mosquitoes. Outbreaks of Zika virus in Brazil during the 2016 Olympics in Rio de Janeiro focused worldwide attention on the disease.

Infection with Zika virus usually causes mild or no symptoms but can lead to several neurological conditions, including microcephaly (small head) in newborns. The virus was isolated in 1947 from a monkey in Africa and was found in humans in 1952, but prior to

2007, only a few cases of Zika virus infection were recorded. Major epidemics occurred in the Pacific Islands in 2013. In 2015, epidemics of Zika virus infection erupted in Brazil and then spread to other parts of South America, Central America, the Caribbean, and North America. Although transmitted primarily by mosquito vectors, the virus is present in human body fluids and can be transmitted sexually.

The Zika virus genome consists of one single-stranded RNA molecule consisting of about 10,800 nucleotides. It contains a single gene, which is translated and processed into 10 different proteins. A characteristic of Zika virus is a high rate of recombination within its genome, which is uncommon in other flaviviruses and has perhaps contributed to its rapid spread around the globe. The virus has undergone multiple recombination events since it was first discovered. Zika virus undergoes rapid evolution, and a number of different strains have evolved.

Our understanding of Zika origin and spread has been greatly facilitated by studies that have isolated, sequenced, and analyzed the genomes of Zika viruses from around the world. For example, studies of Zika genomes indicate that the virus first evolved into two different lineages, one from Africa and another from Asia. Based on the number of genetic differences between them, researchers have estimated that the African and Asian strains diverged sometime in the seventeenth century. The outbreaks in the Pacific Islands and the Americas emerged from the Asian lineage. Genetic analysis

demonstrated that hundreds of infections in Florida originated from at least 4 and perhaps as many as 40 separate introductions, mostly from countries and territories in the Caribbean.

CONCEPTS

Influenza is caused by RNA viruses. New strains of influenza viruses appear through antigenic shift, in which new viral genomes are created through the reassortment of RNA molecules of different strains. Rhinoviruses are small RNA viruses that often cause respiratory infections. Zika virus is an RNA virus that has spread rapidly around the world in recent years.

Chapter 9 Review

CHAPTER SUMMARY

Section 9.1 Learning Objective: Be able to list some of the reasons that the study of bacteria and viruses is important.

- Bacteria and viruses play important roles in human health and society and in the world ecosystem. They are extremely abundant and make up the majority of life's diversity. Analysis of DNA sequences allow study of bacteria that cannot be cultured in the laboratory.

Section 9.2 Learning Objective: Be able to describe how bacteria are cultured and studied in the laboratory.

- Bacteria and viruses are well suited to genetic studies: they are small, have a small haploid genome, undergo rapid reproduction, and produce large numbers of progeny through asexual reproduction.
- The bacterial genome normally consists of a single circular molecule of double-stranded DNA. Plasmids are small pieces of bacterial DNA that can replicate independently of the bacterial chromosome.

Section 9.3 Learning Objective: Be able to summarize the primary mechanisms by which bacteria exchange genes.

- DNA can be transferred between bacteria by conjugation, transformation, or transduction.
- Conjugation is the transfer of genetic material from one bacterial cell to another by direct contact between the two cells. Conjugation is controlled by an episome called the F factor. The time it takes for individual genes to be transferred during conjugation provides information about the order of the genes, and the distances between them, on the bacterial chromosome.
- Bacteria take up DNA from the environment through the process of transformation. Frequencies of cotransformation provide information about the physical distances between chromosomal genes.
- Complete genome sequences of many bacterial species have been determined. This sequence information indicates that horizontal gene transfer—the movement of DNA between species—is common in bacteria.

Section 9.4 Learning Objective: Be able to describe the different ways that bacteria avoid infection by viruses and other foreign DNA elements.

- Bacteria and archaea have evolved mechanisms to prevent the entry and reproduction of viruses and other invading DNA. These include systems that prevent viral attachment and injection of the viral DNA or RNA into the cell, restriction-modification systems, and CRISPR-Cas immunity.

- CRISPR-Cas systems provide adaptive immunity, the ability to recognize and remember DNA from specific pathogens. In response, viruses have evolved mechanisms to avoid bacterial defenses.

Section 9.5 Learning Objective: Be able to describe the genetic systems of viruses.

- Viruses are replicating structures with DNA or RNA genomes that may be double stranded or single stranded and linear or circular.
- Bacterial genes may be incorporated into phage coats and transferred to other bacteria by phages in a process called transduction. Rates of cotransduction can be used to map bacterial genes.
- Phage genes can be mapped by infecting bacterial cells with two different phage strains and counting the recombinant plaques produced by the progeny phages.
- A number of viruses have RNA genomes. Retroviruses encode reverse transcriptase, an enzyme used to make a DNA copy of the viral genome, which then integrates into the host genome as a provirus. HIV is a retrovirus that is the causative agent for AIDS.
- Influenza is caused by RNA viruses. These viruses evolve both through small changes that take place by mutation (antigenic drift) and through major changes that take place via the reassortment of genetic material from different strains within a host (antigenic shift).

- Rhinoviruses are small RNA viruses that often cause respiratory infections.
- Zika virus is an RNA virus that has rapidly spread around the world.

WORKED PROBLEMS

Problem 1

DNA from a strain of bacteria with genotype $a^+ b^+ c^+ d^+ e^+$ was isolated and used to transform a strain of bacteria that was $a^- b^- c^- d^- e^-$. The transformants were tested for the presence of donated genes. The following genes were cotransformed:

a^+ and d^+
b^+ and e^+
c^+ and d^+
c^+ and e^+

What is the order of genes *a*, *b*, *c*, *d*, and *e* on the bacterial chromosome?

» Solution Strategy

What information is required in your answer to the problem?

The order of genes *a*, *b*, *c*, *d*, and *e* on the bacterial chromosome.

What information is provided to solve the problem?

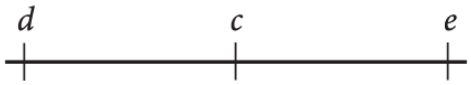
- The donor cells were a^+ b^+ c^+ d^+ e^+ and the recipient cells were a^- b^- c^- d^- e^-.
- The combinations of genes that were cotransformed.

For help with this problem, review:

Transformation in Bacteria in Section 9.3.

» Solution Steps

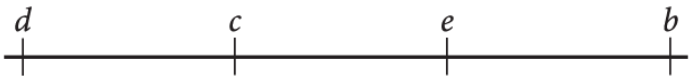
In this transformation experiment, gene c^+ is cotransformed with both gene e^+ and gene d^+, but genes e^+ and d^+ are not cotransformed; therefore, the *c* locus must be between loci *d* and *e*:



Recall: The rate at which genes are cotransformed is inversely proportional to the distance between them: genes that are close together are frequently cotransformed, whereas genes that are far apart are rarely cotransformed.

Gene e^+ is also cotransformed with gene b^+, so the *e* and *b* loci must be located close together. Locus *b* could be on either side of locus *e*. To determine whether locus *b* is on the same side of *e* as locus *c*, we

look to see whether genes b^+ and c^+ are cotransformed. They are not, so locus *b* must be on the opposite side of *e* from *c*:



Gene a^+ is cotransformed with gene d^+, so the *a* and *d* loci must be located close together. If locus *a* were located on the same side of *d* as locus *c*, then genes a^+ and c^+ would be cotransformed. Because these genes display no cotransformation, locus *a* must be on the opposite side of locus *d*:

Problem 2

Consider three genes in *E. coli*: thr^+, ara^+, and leu^+ (which give the cell the ability to synthesize threonine, arabinose, and leucine, respectively). All three of these genes are close together on the *E. coli* chromosome. Phages are grown in a thr^+ ara^+ leu^+ strain of bacteria (the donor strain). The phage lysate is collected and used to infect a strain of bacteria that is thr^- ara^- leu^-. The recipient bacteria are then tested on a selective medium lacking leucine. Bacteria that grow and form colonies on this medium (leu^+ transductants) are then replica-plated on a medium lacking threonine and on a medium lacking arabinose to see which are thr^+ and which are ara^+.

Another group of the recipient bacteria are tested on a medium lacking threonine. Bacteria that grow and form colonies on this medium (*thr*$^+$ transductants) are then replica-plated on a medium lacking leucine and on a medium lacking arabinose to see which are *ara*$^+$ and which are *leu*$^+$. Results from these experiments are as follows:

Selected gene	Cells with cotransduced genes (%)
leu$^+$	3 *thr*$^+$
	76 *ara*$^+$
thr$^+$	3 *leu*$^+$
	0 *ara*$^+$

How are the loci arranged on the chromosome?

» Solution Strategy

What information is required in your answer to the problem?

The order of genes *thr, ara,* and *leu* on the bacterial chromosome.

What information is provided to solve the problem?

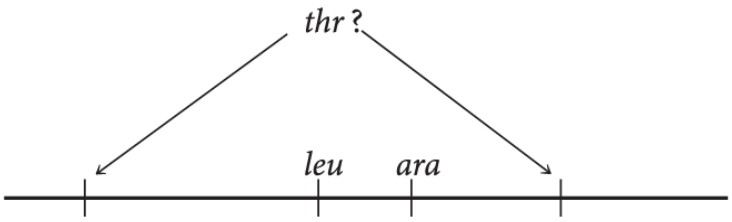
- The genes are located close together on the *E. coli* chromosome.
- The donor strain is *thr*$^+$ *ara*$^+$ *leu*$^+$ and the recipient strain is *hr*$^-$ *ara*$^-$ *leu*$^-$.
- The percentage of cells with cotransduced genes.

For help with this problem, review:

Transduction in Section 9.5.

» Solution Steps

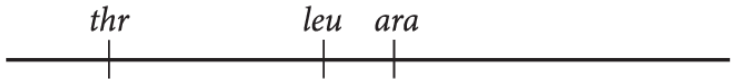
Notice that when we select for *leu*$^+$ (the top half of the table), most of the selected cells are also *ara*$^+$. This finding indicates that the *leu* and *ara* genes are usually cotransduced and are therefore located close together. In contrast, *thr*$^+$ is only rarely cotransduced with *leu*$^+$, indicating that *leu* and *thr* are much farther apart. On the basis of these observations, we know that *leu* and *ara* are closer together than are *leu* and *thr*, but we don't yet know the order of the three genes—whether *thr* is on the same side of *ara* as *leu* or on the opposite side:



Description
Genes l e u and a r a are in the middle of the map, but on the right and left sides are 2 blank notches. Above the line map is a t h r and a question mark. From this point, 2 arrows are drawn down pointing to the 2 blank notches in the map.

Hint: Genes located close together are more likely to be cotransduced than are genes located far apart.

Notice that although the cotransduction frequency for *thr* and *leu* is 3%, no thr^+ ara^+ cotransductants are observed. This finding indicates that *thr* is closer to *leu* than to *ara*, and therefore *thr* must be on the opposite side of *leu* from *ara*:



Hint: We can determine the position of *thr* with respect to the other two genes by looking at the cotransduction frequencies when thr^+ is selected.

IMPORTANT TERMS

prototrophic
minimal medium
auxotroph
complete medium
colony
plasmid
episome
F (fertility) factor
conjugation
transformation
transduction
pilus
human microbiome
competent

transformant
cotransformed
horizontal gene transfer
restriction-modification system
CRISPR-Cas system
CRISPR array
Cas proteins
protospacer-adjacent motif (PAM)
virus
virulent phage
temperate phage
prophage
plaque
generalized transduction
specialized transduction
transducing phage
transductant
cotransduced
retrovirus
reverse transcriptase
provirus
integrase
oncogene
antigenic drift
antigenic shift

ANSWERS TO CONCEPT CHECKS

1. d
2. b
3. a
4. *gal*
5. *his* and *leu*
6. b
7. c
8. Reverse transcriptase

Chapter 9 Assessment

COMPREHENSION QUESTIONS

Section 9.1

1. What are some advantages of using bacteria and viruses for genetic studies?

Section 9.2

2. Explain how auxotrophic bacteria are isolated.
3. What is the difference between a complete medium and a minimal medium? How are complete media and minimal media to which one or more nutrients have been added (selective media) used to isolate auxotrophic mutants of bacteria?

Section 9.3

4. Briefly explain the differences between F^+, F^-, Hfr, and F' cells.
5. What types of matings are possible between F^+, F^-, Hfr, and F' cells? What outcomes do these matings produce? What is the role of the F factor in conjugation?

6. Explain how interrupted conjugation, transformation, and transduction can be used to map bacterial genes. How are these methods similar, and how are they different?
7. What is horizontal gene transfer, and how might it take place?

Section 9.4

8. What are the three stages by which CRISPR-Cas immunity occurs? What takes place in each stage?
9. What problem is solved by the requirement that a PAM sequence be adjacent to a protospacer in order to be recognized by CRISPR-Cas systems?

Section 9.5

10. List some of the characteristics that make bacteriophages ideal organisms for many types of genetic studies.
11. What types of genomes do viruses have?
12. Briefly describe the differences between the lytic cycle of virulent phages and the lysogenic cycle of temperate phages.
13. Briefly explain how genes in phages are mapped.
14. Briefly describe the genetic structure of a typical retrovirus.
15. Explain how a retrovirus, which has an RNA genome, is able to integrate its genetic material into that of a host having a DNA genome.
16. What are the evolutionary origins of HIV-1 and HIV-2?
17. Most humans are not easily infected by avian influenza. How, then, do DNA sequences from avian influenza become incorporated into human influenza?

APPLICATION QUESTIONS AND PROBLEMS

Section 9.1

18. *Suppose you want to compare the species of bacteria that exist in a polluted stream with the species that exist in an unpolluted stream. Traditionally, bacteria have been identified by growing them in the laboratory and comparing their physical and biochemical properties. You recognize that you will be unable to culture most of the bacteria that reside in the streams. How might you go about identifying the species in the two streams without culturing them in the laboratory?

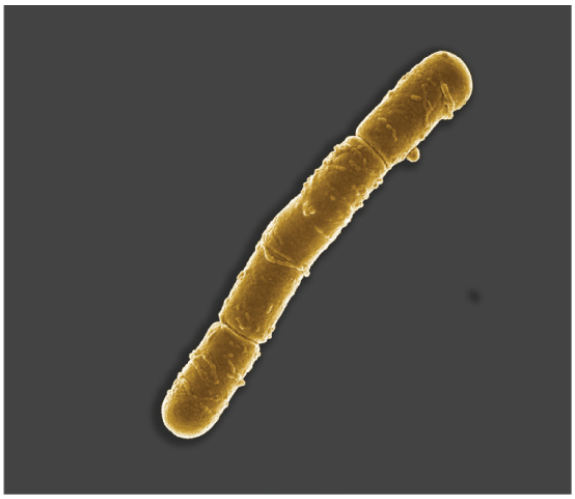
Section 9.3

19. John Smith is a pig farmer. For the past five years, Smith has been adding vitamins and low doses of antibiotics to his pig food; he says that these supplements enhance the growth of the pigs. Within the past year, however, several of his pigs died from infections of common bacteria, which failed to respond to large doses of antibiotics. Can you explain the increased rate of mortality due to infection in Smith's pigs? What advice might you offer Smith to prevent this problem in the future?
20. Rarely, the conjugation of Hfr and F^- cells produces two Hfr cells. Explain how this event takes place.

21. In **Figure 9.8**, what do the red and blue parts of the DNA labeled by balloon 6 represent?
22. DNA from a strain of *Bacillus subtilis* with the genotype trp^+ tyr^+ was used to transform a recipient strain with the genotype trp^- tyr^-. The following numbers of transformed cells were recovered:

Genotype	Number of transformed cells
trp^+ tyr^-	**154**
trp^- tyr^+	**312**
trp^+ tyr^+	**354**

What do these results suggest about the linkage of the *trp* and *tyr* genes?



Nano Creative/Science Source.

B. subtilis.

23. *DNA from a strain of *B. subtilis* with genotype $a^+ b^+ c^+ d^+ e^+$ is used to transform a strain with genotype $a^- b^- c^- d^- e^-$. Pairs of genes are checked for cotransformation, and the following results are obtained:

Pair of genes	Cotransformation	Pair of genes	Cotransformation
a^+ and b^+	No	b^+ and d^+	No
a^+ and c^+	No	b^+ and e^+	Yes
a^+ and d^+	Yes	c^+ and d^+	No
a^+ and e^+	Yes	c^+ and e^+	Yes
b^+ and c	Yes	d^+ and e^+	No

On the basis of these results, what is the order of the genes on the bacterial chromosome?

24.

Rollin Hotchkiss and Julius Marmur studied transformation in the bacterium *Streptococcus pneumoniae* (R. D. Hotchkiss and J. Marmur. 1954. *Proceedings of the National Academy of Sciences of the United States of America* 40:55–60). They examined four mutations in this bacterium: penicillin resistance (*P*), streptomycin resistance (*S*), sulfanilamide resistance (*F*), and the ability to use mannitol (*M*). They extracted DNA from strains of bacteria with different combinations of different mutations and used this DNA to transform wild-type bacterial cells ($P^+ S^+ F^+ M^+$). The results from one of their transformation experiments are shown here:

Donor DNA	Recipient DNA	Transformants	Percentage of all cells
$M\ S\ F$	$M^+\ S^+\ F^+$	$M^+\ S\ F^+$	4.0
		$M^+\ S^+\ F$	4.0
		$M\ S^+\ F^+$	2.6
		$M\ S\ F^+$	0.41
		$M^+\ S\ F$	0.22
		$M\ S^+\ F$	0.0058
		$M\ S\ F$	0.0071

a. Hotchkiss and Marmur noted that the percentage of cotransformation was higher than would be expected on a random basis. For example, the results show that 2.6% of the cells were transformed into *M* and 4% were transformed into *S*. If the *M* and *S* traits were inherited independently, the expected probability of cotransformation of *M* and *S* (*M S*) would be 0.026 × 0.04 = 0.001, or 0.1%. However, they observed 0.41% *M S* cotransformants, four times more than they expected. What accounts for the relatively high frequency of cotransformation of the traits they observed?
b. On the basis of the results, what conclusion can you draw about the order of the *M*, *S*, and *F* genes on the bacterial chromosome?
c. Why is the rate of cotransformation for all three genes (*M S F*) almost the same as the rate of cotransformation for *M F* alone?

25. In the course of a study on the effects of the mechanical shearing of DNA, Eugene Nester, A. T. Ganesan, and Joshua Lederberg studied the transfer, by transformation, of sheared DNA from a wild-type strain of *B. subtilis* (his_2^+ aro_3^+ tyr_2^+ $aro1^+$ $tyr1^+$ $aro2^+$) to strains of bacteria carrying a series of mutations (E. W. Nester, A. T. Ganesan, and J. Lederberg. 1963. *Proceedings of the National Academy of Sciences of the United States of America* 49:61–68). They reported the following rates of cotransformation between his_2^+ and the other genes (expressed as cotransfer rate):

Genes	Rate of cotransfer
his_2^+ and aro_3^+	0.015
his_2^+ and tyr_2^+	0.10
his_2^+ and aro_1^+	0.12
his_2^+ and tyr_1	0.23
his_2^+ and aro_2^+	0.05

On the basis of these data, which gene is farthest from his_2^+? Which gene is closest?

Section 9.4

26. Place the following components of CRISPR-Cas systems in the order in which they function during generation of CRISPR-Cas immunity.
a. Effector complex binds to the foreign DNA

b. Transcription of CRISPR array into a long CRISPR precursor RNA
c. crRNA combines with a Cas protein to form an effector complex
d. Incorporation of spacers into CRISPR array
e. Cas protein in the effector complex cleaves the foreign DNA
f. CRISPR precursor RNA (pre-crRNA) is cleaved and processed into crRNAs

27. CONNECT In Chapter 1, we discussed the theory of the inheritance of acquired characteristics, an idea that was later discredited. Recently some researchers have claimed that the CRISPR-Cas system represents a form of inheritance of acquired characteristics. How might the CRISPR-Cas system be considered a type of inheritance of acquired characteristics? Do you think CRISPR-Cas represents inheritance of acquired characteristic? Why or why not?

Section 9.5

28. *Two mutations that affect plaque morphology in phages (a^- and b^-) have been isolated. Phages carrying both mutations a^- b^- are mixed with wild-type phages (a^+ b^+) and added to a culture of bacterial cells. Once the phages have infected and lysed the bacteria, samples of the phage lysate are collected

and cultured on plated bacteria. The following number of plaques are observed:

Plaque phenotype	Number
$a^+ b^+$	2043
$a^+ b^-$	320
$a^- b^+$	357
$a^- b^-$	2134

What is the frequency of recombination between the *a* and *b* genes?

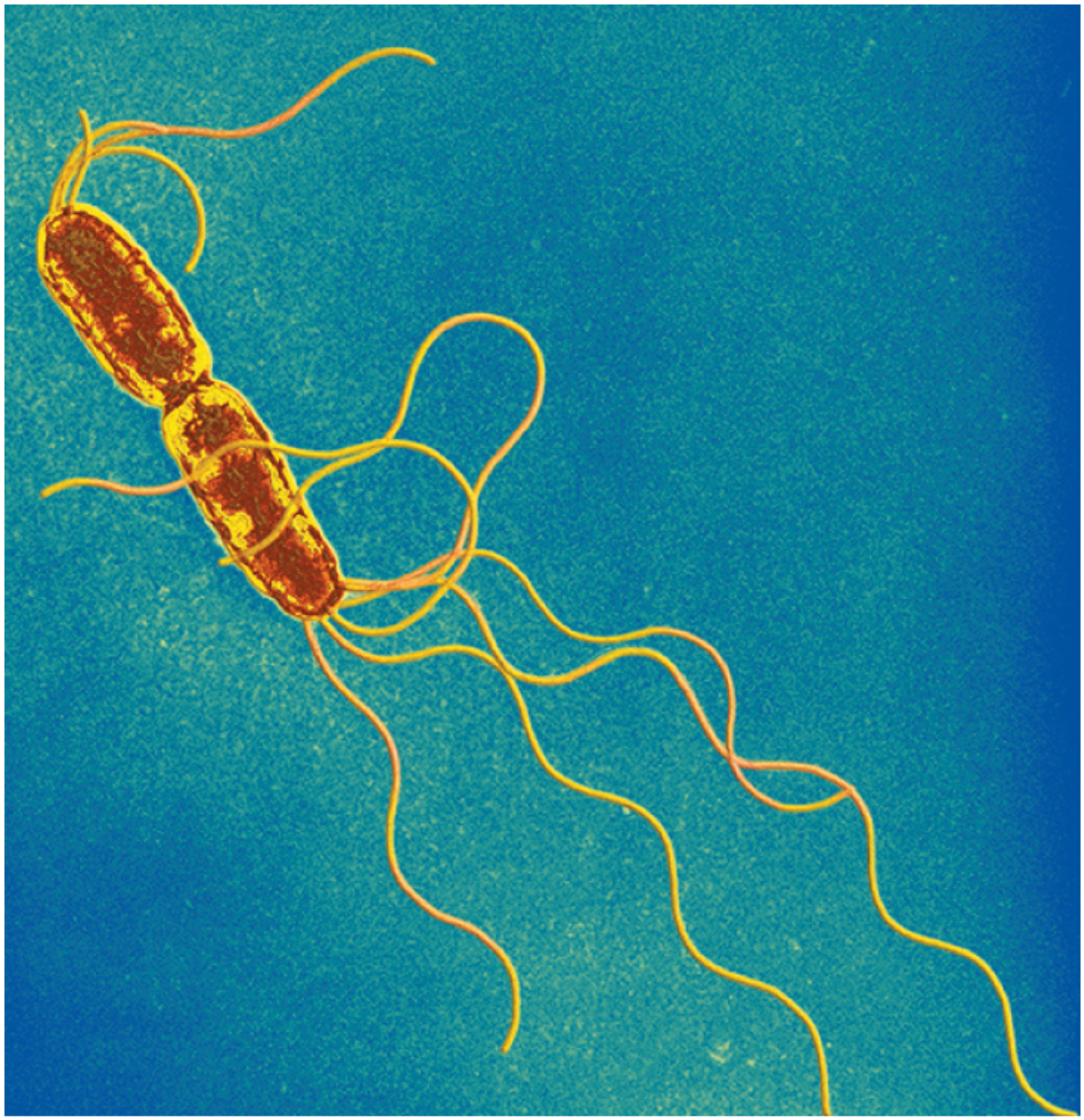
29. *

T. Miyake and M. Demerec examined proline-requiring mutations in the bacterium *S. typhimurium* (T. Miyake and M. Demerec. 1960. *Genetics* 45:755–762). On the basis of complementation testing, they found four proline auxotrophs: *proA*, *proB*, *proC*, and *proD*. To determine whether *proA*, *proB*, *proC*, and *proD* loci were located close together on the bacterial chromosome, they conducted a transduction experiment. Bacterial strains that were $proC^+$ and had mutations at *proA*, *proB*, or *proD* were used as donors. The donors were infected with bacteriophages, and progeny phages were allowed to infect recipient bacteria with genotype $proC^- proA^+ proB^+ proD^+$. The recipient bacteria were then plated on a selective medium that allowed only $proC^+$ bacteria to grow. After this, the $proC^+$ transductants were plated on selective media to reveal their

genotypes at the other three *pro* loci. The following results were obtained:

Donor genotype	Transductant genotype	Number
$proC^{+}\ proA^{-}\ proB^{+}\ proD^{+}$	proC+ proA+ proB+ proD+	2765
	$proC^{+}\ proA^{-}\ proB^{+}\ proD^{+}$	3
$proC^{+}\ proA^{+}$ proB− $proD^{+}$	$proC^{+}\ proA^{+}\ proB^{+}\ proD^{+}$	1838
	$proC^{+}\ proA^{+}$ proB− $proD^{+}$	2
$proC^{+}\ proA^{+}\ proB^{+}\ proD^{-}$	$proC^{+}\ proA^{+}\ proB^{+}\ proD^{+}$	1166
	$proC^{+}\ proA^{+}\ proB^{+}\ proD^{-}$	0

a. Why are there no proC− genotypes among the transductants?
b. Which genotypes represent single transductants, and which represent cotransductants?
c. Is there evidence that *proA*, *proB*, and *proD* are located close to *proC*? Explain your answer.



Kwangshin Kim/Science Source.

S. typhimurium.

30. *A geneticist isolates two mutations in a bacteriophage. One mutation causes clear plaques (*c*), and the other produces minute plaques (*m*). Previous mapping experiments have established that the genes responsible for these two mutations are 8 map units apart. The geneticist mixes phages with

genotype $c^+ m^+$ and genotype $c^- m^-$ and uses the mixture to infect bacterial cells. She collects the progeny phages and cultures a sample of them on plated bacteria. A total of 1000 plaques are observed. What numbers of the different types of plaques ($c^+ m^+$, $c^- m^-$, $c^+ m^-$, $c^- m^+$) should she expect to see?

31. CONNECT The geneticist carries out the same experiment described in Problem 30, but this time she mixes phages with genotypes $c^+ m^-$ and $c^- m^+$. Are the mutations in coupling or repulsion (see Chapter 7)? What results are expected from *this* cross?

32. *A geneticist isolates two bacteriophage *r* mutants (r_{13} and r_2) that cause rapid lysis. He carries out the following crosses and counts the number of plaques listed here:

Genotype of parental phage	Progeny	Number of plaques
$h^+ r_{13}^- \times h^- r_{13}^+$	$h^+ r_{13}^+$	1
	$h^- r_{13}^+$	104
	$h^+ r_{13}^-$	110
	$h^- r_{13}^-$	2
Total		217
$h^+ r_2^- \times h^- r_2^+$	$h^+ r_2^+$	6
	$h^- r_2^+$	86
	$h^+ r_2^-$	81

	$h^- r_2^-$	7
Total		180

a. Calculate the recombination frequencies between r_2 and h and between r_{13} and h.
b. Draw all possible linkage maps for these three genes.

33. CONNECT **E. coli* cells are simultaneously infected with two strains of phage λ. One strain has a mutant host range, is temperature sensitive, and produces clear plaques (genotype *h st c*); another strain carries the wild-type alleles (genotype h^+ st^+ c^+). Progeny phages are collected from the lysed cells and are plated on bacteria. The following number of different progeny phages are obtained:

Progeny phage genotype	Number of plaques
$h^+ c^+ st^+$	321
h c st	338
h^+ c st	26
h c^+ st^+	30
h^+ c st^+	106
h c^+ st	110
h^+ c^+ st	5
h c st^+	6

a. Determine the order of the three genes on the phage chromosome.
b. Determine the map distances between the genes.
c. Determine the coefficient of coincidence and the interference (see pp. 199–201 in Chapter 7).

34. A donor strain of bacteria with alleles $a^+ b^+ c^+$ is infected with phages to map the donor chromosome using generalized transduction. The phage lysate from the bacterial cells is collected and used to infect a second strain of bacteria that are $a^- b^- c^-$. Bacteria with the a^+ allele are selected, and the percentage of cells with cotransduced b^+ and c^+ alleles are recorded.

Donor	Recipient	Selected allele	Cells with cotransduced allele (%)
$a^+ b^+ c^+$	$a^- b^- c^-$	a^+	25 b^+
		a^+	3 c^+

Is gene *b* or gene *c* closer to gene *a*? Explain your reasoning.

35. A donor strain of bacteria with genotype $leu^+ gal^- pro^+$ is infected with phages. The phage lysate from the bacterial cells is collected and used to infect a second strain of bacteria that are $leu^- gal^+ pro^-$. The second strain is selected for leu^+, and the following cotransduction data are obtained:

Donor	Recipient	Selected allele	Cells with cotransduced allele (%)
$leu^+ gal^-$	$leu^- gal^+$	leu^+	47 pro^+

pro$^+$	*pro*$^-$		
		leu$^+$	26 *gal*–

Which genes are closest, *leu* and *gal* or *leu* and *pro*?

36. Viruses from which organism contributed the most RNA to the H1N1 influenza virus shown at the bottom of **Figure 9.28**?

CHALLENGE QUESTION

Section 9.3

37. A group of genetics students mix two auxotrophic strains of bacteria: one is *leu*$^+$ *trp*$^+$ *his*– *met*$^-$ and the other is *leu*$^-$ *trp*$^-$ *his*$^+$ *met*$^+$. After mixing the two strains, they plate the bacteria on a minimal medium and observe a few prototrophic colonies (*leu*$^+$ *trp*$^+$ *his*$^+$ *met*$^+$). They assume that some gene transfer has taken place between the two strains. How can they determine whether the transfer of genes is due to conjugation, transduction, or transformation?

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 9.1

1. Luiz Roesch and colleagues (Roesch L. F., R. R. Fulthorpe, A. Riva, et al. 2007. *ISME Journal* 1:283–290) conducted a study to determine how many types of bacteria exist in a gram of soil at different locations around the world. They found that soil from agricultural fields harbored considerably fewer types of bacteria than did soil from forests (see Life in a Bacterial World). Propose some possible reasons that agricultural fields might have less bacterial diversity than forests.

Section 9.2

2. One advantage of using bacteria and viruses for genetic study is the fact that they have haploid genomes. Explain why a haploid genome facilitates genetic analysis.

Section 9.3

3. Antibiotic resistance genes are often found on R plasmids (see Natural Gene Transfer and Antibiotic Resistance). A likely source of the R plasmids is bacteria that produce the antibiotic. Why would some bacteria produce antibiotics (chemicals that kill bacteria), and why would they carry R plasmids?
4. Bacteria have evolved numerous mechanisms to prevent the invasion of foreign viral DNA (see Bacterial Defense Mechanisms). Yet clearly many bacteria have evolved competence, the ability to take up foreign DNA from the environment. Why do bacteria take up naked DNA from the environment and yet exclude DNA from viruses?

Section 9.5

5. Researchers have recently discovered giant viruses that are 1 μm in length, the same size as some bacterial cells. The genomes of these viruses contain over 2 million base pairs of DNA, which is more DNA than is found in many bacterial genomes, and their genomes contain hundreds—in some cases, thousands—of genes. Given these observations and what you know about viruses, should viruses be considered living or nonliving? Give arguments for and against considering viruses as living organisms.
6. RNA viruses often undergo rapid evolution. What aspects of their biology contribute to their high rate of evolution? What are some consequences of their rapid evolution?

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Draw a concept map that outlines the relationship of leprosy and the bacterium *Mycobacterium leprae*. Include how genome sequences of medieval *M. leprae* are related to those in modern strains of *M. leprae*.

Sections 9.3 and 9.5

2. Create a concept map that displays the interrelationships among the following terms: bacterial gene exchange, conjugation, transduction, transformation, F factor, sex pilus, F^+ cells, F^- cells, Hfr cells, F' cells, competent, transformants, horizontal gene transfer, generalized transduction, specialized transformation, transducing phage, transductants.

Section 9.4

3. Draw a concept map that outlines the different components and functions of CRISPR-Cas systems.

Section 9.5

4. Create a concept map that outlines the relationships among the following terms: retrovirus, reverse transcriptase, cDNA, provirus, integrase, oncogenes, *gag, pol*, and *env* genes.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 10

DNA: The Chemical Nature of the Gene

Eddie Gerald/Alamy Stock Photo.

The Denisova cave in Siberia, where scientists found 90,000-year-old bones from an ancient human. Analysis of DNA from these bones revealed that the woman was a hybrid between a Neanderthal mother and a Denisovan father.

Hominin History in Ancient DNA

Deep in the Altai mountains of Siberia lies the Denisova cave, famous for its accumulation of fossils and animal remains. In 2018, scientists extracted DNA from a 90,000-year-old human bone found in the cave and sequenced its owner's genome. Analysis revealed that there were two copies of sequences located on the X chromosome, indicating that the bone came from a female. The thickness of the bone suggested that she was at least 13 years old at the time of her death. Researchers named this ancient woman Denny. Remarkably, Denny's DNA showed that she was a hybrid between two now-extinct groups of early humans (hominins), the result of a mating between a Neanderthal woman and a Denisovan man.

Denisovans were first discovered in 2009, when scientists found a single 40,000-year-old finger bone in the Denisova cave. Although the bone was too fragmentary to draw any conclusion about its origin based on anatomy, scientists successfully extracted DNA from it. They were able to sequence and reconstruct the person's entire genome from that extracted DNA. Study of this genome revealed that the bone came from a new hominin, related to Neanderthals but distinct from Neanderthals and other known hominins. These people were given the name Denisovans, after the cave from which the bone came. Researchers have since found traces of Denisovan DNA in some present-day humans in Southeast Asia, suggesting that early *Homo sapiens* and Denisovans also interbred. Both Neanderthals and Denisovans have long since

disappeared, perhaps outcompeted or exterminated by *Homo sapiens* some 40,000 years ago.

The researchers who examined Denny's genome found that 39% of her DNA came from Neanderthals and 42% came from Denisovans, demonstrating that both species contributed equally to her ancestry. Further analysis revealed that Denny possessed a level of heterozygosity expected if she had inherited one set of chromosomes from a Neanderthal mother and the other set from a Denisovan father. The data were unequivocal: Denny was an F_1 hybrid between the two species. Examination revealed that Denny's Denisovan father possessed some Neanderthal DNA himself, indicating that earlier matings between Denisovans and Neanderthals took place in Denny's distant ancestors.

DNA, with its double-stranded spiral, is among the most elegant of all biological molecules, but the double helix is not just a beautiful structure; it also gives DNA incredible stability and permanence, as evidenced by our ability to extract and sequence DNA from a 90,000-year-old bone, revealing startlingly intimate details of ancient family life. In an even more incredible feat, geneticists in 2013 sequenced the genome of a long-extinct ancestral horse from DNA extracted from a 700,000-year-old bone fragment recovered from the permafrost of Yukon, Canada.

THINK-PAIR-SHARE

What do you think might be some of the problems associated with isolating and sequencing DNA from ancient samples, such as that of the 90,000-year-old bone from the Denisova cave?

This chapter focuses on how DNA was identified as the source of genetic information and on how it encodes the genetic instructions for all life. We begin by considering the basic requirements for the genetic material and the history of the study of DNA—how its relation to genes was uncovered and its structure determined. The history of DNA illustrates several important points about the nature of scientific research. As with so many important scientific advances, the structure of DNA and its role as the genetic material were not discovered by any single person but were gradually revealed over a period of almost 100 years, thanks to the work of many investigators. Our understanding of the relation between DNA and genes was enormously enhanced in 1953, when James Watson and Francis Crick, analyzing data provided by Rosalind Franklin and Maurice Wilkins, proposed a three-dimensional structure for DNA that brilliantly illuminated its role in genetics.

After reviewing the discoveries that led to our current understanding of DNA, we examine DNA structure. While the structure of DNA is important in its own right, the key genetic concept is the relation between the structure and the function of DNA—that is, how its structure allows it to serve as the genetic material.

10.1 Genetic Material Possesses Several Key Characteristics

Life is characterized by tremendous diversity, but the coding instructions for all living organisms are written in the same genetic language—that of nucleic acids. Surprisingly, the idea that genes are made of nucleic acids was not widely accepted until after 1950. This skepticism was due in part to a lack of knowledge about the structure of deoxyribonucleic acid (DNA). Until the structure of DNA was understood, no one knew how DNA could store and transmit genetic information.

Even before nucleic acids were identified as the genetic material, biologists recognized that whatever the nature of the genetic material, it must possess four important characteristics:

1. **The genetic material must contain complex information.** First and foremost, the genetic material must be capable of storing large amounts of information—instructions for the traits and functions of an organism.
2. **The genetic material must replicate faithfully.** Every organism begins life as a single cell. To produce a complex multicellular organism like yourself, this single cell must undergo billions of cell divisions. At each cell division, the genetic instructions must be accurately transmitted to descendant cells. And when

organisms reproduce and pass genes to their progeny, the genetic instructions must be copied with fidelity.

3. **The genetic material must encode the phenotype.** The genetic material (the genotype) must have the capacity to be expressed as a phenotype—to code for traits. The product of a gene is often a protein or an RNA molecule, so there must be a mechanism for genetic instructions in the DNA to be copied into RNAs and proteins.
4. **The genetic material must have the capacity to vary.** Genetic information must have the ability to vary, because different species—and even individual members of the same species—differ in their genetic makeup.

THINK-PAIR-SHARE Question 1

CONCEPTS

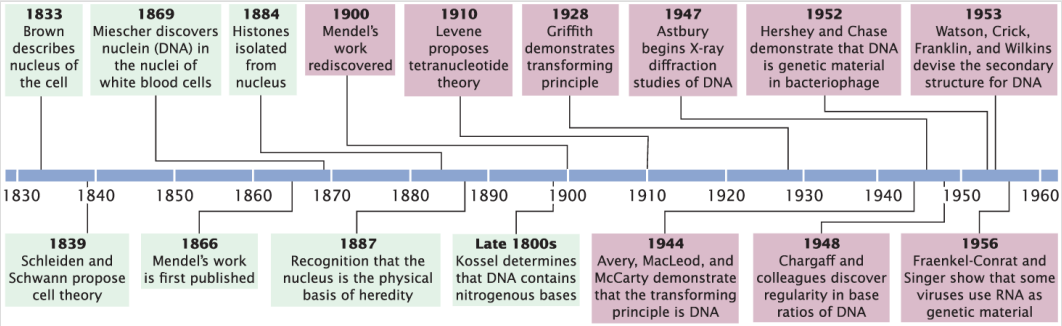
The genetic material must carry large amounts of information, replicate faithfully, express its coding instructions as phenotypes, and have the capacity to vary.

✔ CONCEPT CHECK 1

Why was the discovery of the structure of DNA so important for understanding genetics?

10.2 All Genetic Information Is Encoded in the Structure of DNA or RNA

Although our understanding of how DNA encodes genetic information is relatively recent, the study of DNA structure stretches back more than 100 years (Figure 10.1).



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10.1 Many people have contributed to our understanding of the structure of DNA.

Description

Data from the timeline is as follows from left to right:

18 33, Brown describes nucleus of the cell.

18 39, Schleiden and Schwann propose cell theory.

18 66, Mendel's work is first published.

18 69, Miescher discovers nuclein (D N A) in the nuclei of white blood cells.

18 84, histones isolated from the nucleus.

18 87, recognition that the nucleus is the physical basis of heredity.

Late 18 80's, Kossel determines that D N A contains nitrogenous bases.

19 00, Mendel's work rediscovered.

19 10, Levene proposes tetranucleotide theory.

19 28, Griffith demonstrates transforming principle.

19 44, Avery, MacLeod, and McCarty demonstrate that the transforming principle is D N A.

19 47, Ashbury begins X-ray diffraction studies of D N A.

19 48, Chargaff and colleagues discover regularity in base ratios of D N A.

19 52, Hershey and Chase demonstrate that D N A is genetic material in bacteriophage.

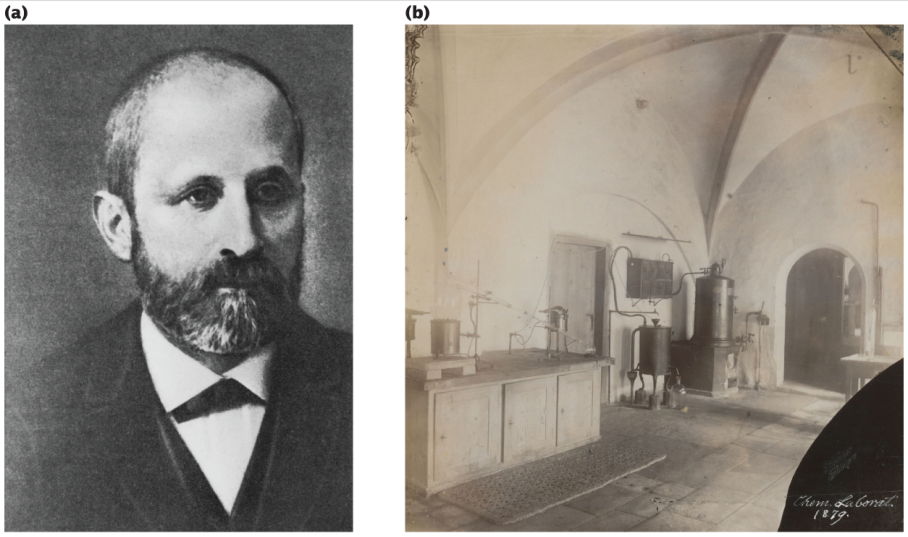
19 53, Watson and Crick devise the secondary structure for D N A.

19 56, Fraenkel-Conrat and Singer show that some viruses use R N A as their genetic material.

Early Studies of DNA

In 1868, Johann Friedrich Miescher (Figure 10.2) graduated from medical school in Switzerland. Influenced by an uncle who believed that the key to understanding disease lay in the chemistry of tissues, Miescher traveled to Tübingen, Germany, to study under Ernst Felix Hoppe-Seyler, an early leader in the emerging field of biochemistry. Under Hoppe-Seyler's direction, Miescher turned his attention to the chemistry of pus, a substance of clear medical importance. Pus contains white blood cells, which have large nuclei, and Miescher developed a method for isolating these nuclei. The minute amounts of nuclear material that he obtained were insufficient for a thorough

chemical analysis, but he did establish that the nuclear material contained a novel substance that was slightly acidic and high in phosphorus. This material, which we now know must have consisted of DNA and protein, Miescher called *nuclein*. The substance was later renamed *nucleic acid* by one of his students.

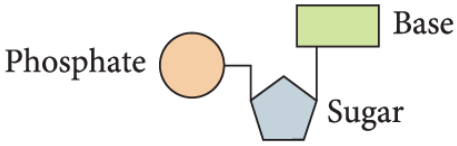


Part a: SPL/Science Source. Part b: Courtesy of the University of Tübingen Library Image Database, Tübingen, Federal Republic of Germany.

10.2 Johann Friedrich Miescher performed the first chemical analysis of DNA.
(a) Portrait of Miescher. (b) Miescher's laboratory in Tübingen, Germany.

By 1887, several researchers had independently concluded that the physical basis of heredity lies in the nucleus. Chromatin was shown to consist of nucleic acid and proteins, but which of these substances was actually the genetic information was not clear. In the late 1800s, Albrecht Kossel carried out further work on the chemistry of DNA and determined that it contains four nitrogenous bases: adenine, cytosine, guanine, and thymine (abbreviated A, C, G, and T).

In the early twentieth century, the Rockefeller Institute in New York City became a center for nucleic acid research. Phoebus Aaron Levene joined the Institute in 1905 and spent the next 40 years studying the chemistry of DNA. He discovered that DNA consists of a large number of linked, repeating units, called **nucleotides**; each nucleotide contains a sugar, a phosphate, and a base.



Nucleotide

Description
The sugar is represented by a pentagon. A line emerges from C 1 of the sugar and is bonded to a rectangle labeled base. A line emerges from C 4 of the sugar and is bonded to a circle labeled phosphate.

Levene incorrectly proposed that DNA consists of a series of four-nucleotide units, each containing all four bases—adenine, guanine, cytosine, and thymine—in a fixed sequence. This concept, known as the tetranucleotide hypothesis, implied that the structure of DNA was not variable enough to make it the genetic material. The tetranucleotide hypothesis contributed to the idea that protein is the genetic material because the structure of protein, with its 20 different amino acids, could be highly variable.

As additional studies of the chemistry of DNA were completed in the 1940s and 1950s, the notion of DNA as a simple, invariant molecule

began to change. Erwin Chargaff and his colleagues carefully measured the amounts of the four bases in DNA from a variety of organisms, and they found that DNA from different organisms varies greatly in base composition. This finding disproved the tetranucleotide hypothesis. They discovered that within each species, there is some regularity in the ratios of the bases: the amount of adenine is always equal to the amount of thymine (A = T), and the amount of guanine is always equal to the amount of cytosine (G = C) (Table 10.1). These findings became known as Chargaff's rules. However, the cause of these ratios among the bases was unknown at the time.

TABLE 10.1 Base composition (percent*) of DNA from different sources and ratios of bases

					Ratio		
Source of DNA	**A**	**T**	**G**	**C**	**A/T**	**G/C**	**(A + G)/(T + C)**
E. coli	26.0	23.9	24.9	25.2	1.09	0.99	1.04
Yeast	31.3	32.9	18.7	17.1	0.95	1.09	1.00
Sea urchin	32.8	32.1	17.7	18.4	1.02	0.96	1.00
Rat	28.6	28.4	21.4	21.5	1.01	1.00	1.00
Human	30.3	30.3	19.5	19.9	1.00	0.98	0.99

*Percent in moles of nitrogenous constituents per 100 g-atoms of phosphate in hydrolysate corrected for 100% recovery. Data from: E. Chargaff and J. Davidson eds., *The Nucleic Acids,* vol 1. (New York: Academic Press, 1955)

CONCEPTS

Details of the composition of DNA were worked out by a number of scientists. At first, DNA was interpreted as being too regular in structure to carry genetic information, but by the 1940s, DNA from different organisms was shown to vary in its base composition.

✔ CONCEPT CHECK 2

What contribution did Levene make to our understanding of DNA structure?

a. He determined that the nucleus contains DNA.
b. He determined that DNA contains four nitrogenous bases.
c. He determined that DNA consists of nucleotides.
d. He determined that the nitrogenous bases of DNA are present in regular ratios.

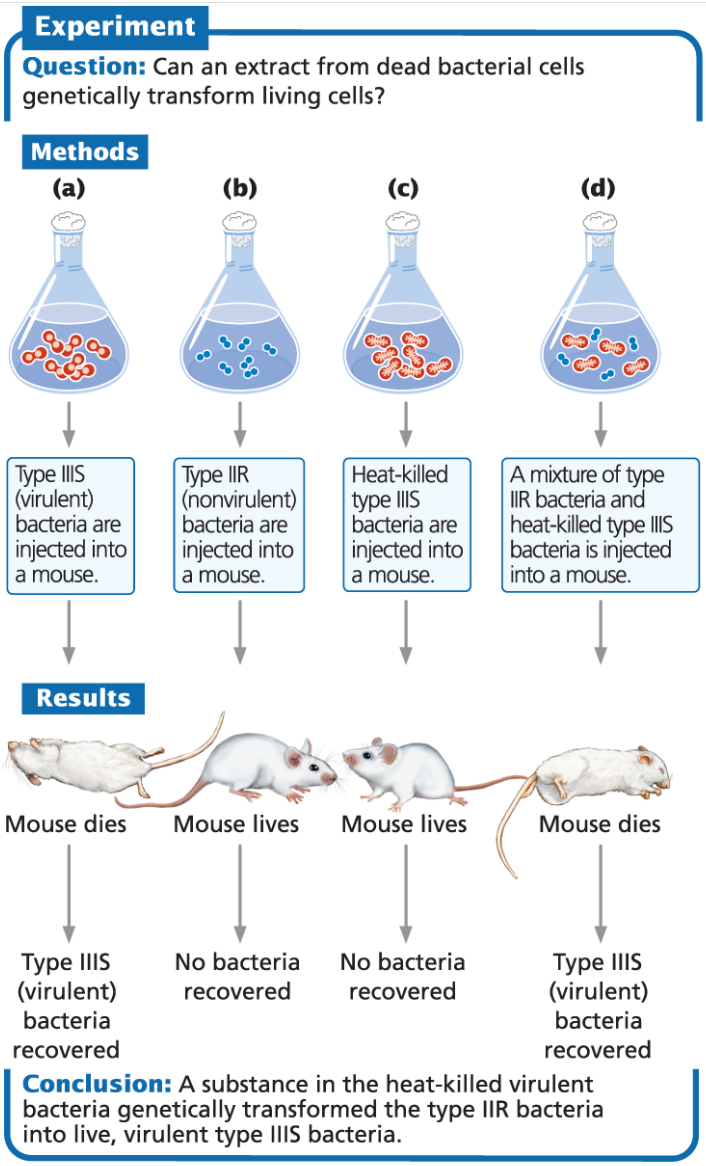
DNA as the Source of Genetic Information

While chemists were working out the structure of DNA, biologists were attempting to identify the carrier of genetic information. Mendel identified the basic rules of heredity in 1866, but he had no idea about the physical nature of hereditary information. By the early 1900s, biologists had concluded that genes resided on chromosomes, which were known to contain both DNA and protein. Two sets of experiments, one conducted on bacteria and the other on viruses, provided pivotal evidence that DNA, rather than protein, was the genetic material.

THE DISCOVERY OF THE TRANSFORMING PRINCIPLE

An initial step in identifying DNA as the source of genetic information came with the discovery of a phenomenon called *transformation* (described in Section 9.3). This phenomenon was first observed in 1928 by Fred Griffith, an English physician whose special interest was the bacterium that causes pneumonia: *Streptococcus pneumoniae*. Griffith had succeeded in isolating several different strains of *S. pneumoniae* (type I, II, III, and so forth). In the virulent (disease-causing) forms of a strain, each bacterium is surrounded by a polysaccharide coat, which makes the bacterial colony appear smooth (S) when grown on an agar plate. Griffith found that these virulent forms occasionally mutated to nonvirulent forms, which lack a polysaccharide coat and produce a rough-appearing colony (R).

Griffith observed that small amounts of living type IIIS bacteria injected into mice caused the mice to develop pneumonia and die; when he examined the dead mice, he found large amounts of type IIIS bacteria in their blood (**Figure 10.3a**). When Griffith injected type IIR bacteria into mice, the mice lived, and no bacteria were recovered from their blood (**Figure 10.3b**). Griffith knew that boiling killed all bacteria and destroyed their virulence; when he injected large amounts of heat-killed type IIIS bacteria into mice, the mice lived, and no type IIIS bacteria were recovered from their blood (**Figure 10.3c**).



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10.3 Griffith's experiments demonstrated transformation in bacteria.

Description

The illustration shows the question as follows: Can an extract from dead bacterial cells genetically transform living cells? The methods section below shows part a where a conical flask has Type 3 S (virulent) bacteria. These bacteria are injected into mice. The results below show that the mouse dies. Eventually, type 3 S (virulent) bacteria are recovered. Part b shows a method where a conical flask has type 2 R (nonvirulent) bacteria. These bacteria are injected into mice. The results below show that the mouse

lives. No bacteria are recovered. Part c shows a method where a conical flask has heat-killed type 3 S bacteria. These bacteria are injected into mice. The results below show that the mouse lives. No bacteria are recovered. Part d shows a method where a conical flask has a mixture of type 2 R bacteria and heat-killed type 3 S bacteria. These bacteria are injected into mice. The results show that the mouse dies. Eventually, type 3 S (virulent) bacteria are recovered. A caption below reads, Conclusion: A substance in the heat-killed virulent bacteria genetically transformed the type 2 R bacteria

into live, virulent type 3 S bacteria.

The results of these experiments were not unusual. However, Griffith got a surprise when he injected his mice with a small amount of living type IIR bacteria along with a large amount of heat-killed type IIIS bacteria. Because both the type IIR bacteria and the heat-killed type IIIS bacteria were nonvirulent, he expected these mice to live. Surprisingly, 5 days after the injections, the mice developed pneumonia and died (**Figure 10.3d**). When Griffith examined blood from the hearts of these mice, he observed live type IIIS bacteria. Furthermore, these bacteria retained their type IIIS characteristics through several generations: their virulence was heritable.

Griffith considered all the possible interpretations of his results. First, there was a possibility that he had not sufficiently sterilized the type IIIS bacteria and thus a few live bacteria had remained in the culture. Any live bacteria injected into the mice would have multiplied and caused pneumonia. Griffith knew that this possibility was unlikely because he had used only heat-killed type IIIS bacteria

in the control experiment, and they never produced pneumonia in the mice.

A second interpretation was that the live type IIR bacteria had mutated to the virulent S form. Such a mutation would cause pneumonia in the mice, but it would produce type IIS bacteria, not the type IIIS that Griffith found in the dead mice. Because type II and type III bacteria differ in a number of traits, many mutations would be required for type II bacteria to mutate to type III bacteria, and the chance of all the mutations occurring simultaneously was impossibly low.

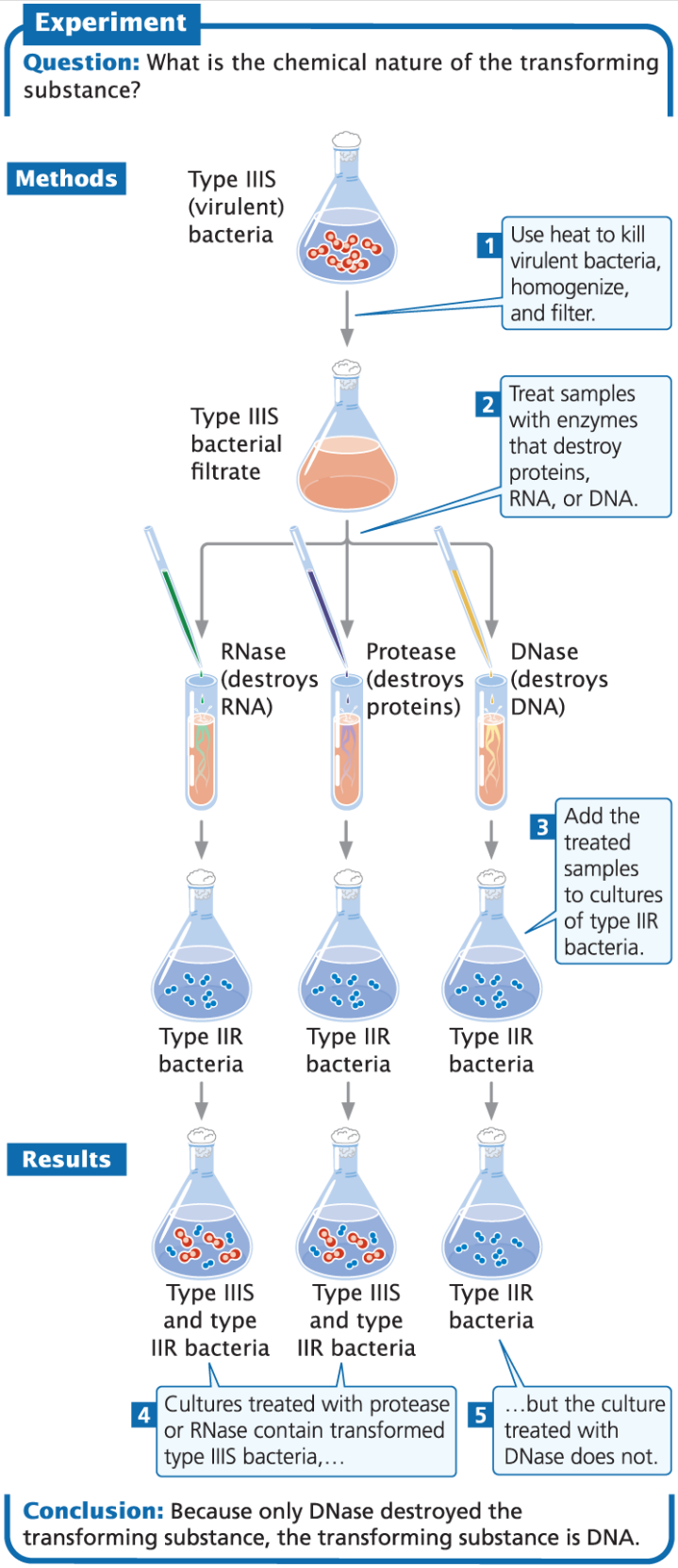
Griffith concluded that the type IIR bacteria had somehow been transformed, acquiring the genetic virulence of the dead type IIIS bacteria, and that this transformation had produced a permanent genetic change in the bacteria. Although Griffith didn't understand the nature of this transformation, he theorized that some substance in the dead bacteria might be responsible. He called this substance the **transforming principle**. › **TRY PROBLEM 19**

IDENTIFICATION OF THE TRANSFORMING PRINCIPLE

At the time of Griffith's report, Oswald Avery (see Figure 10.1) was a microbiologist at the Rockefeller Institute. At first, Avery was skeptical of Griffith's results, but after other microbiologists successfully repeated Griffith's experiments with other bacteria,

Avery set out to understand the nature of the transforming substance.

After 10 years of research, Avery, Colin MacLeod, and Maclyn McCarty succeeded in isolating and partially purifying the transforming substance. They showed that it had a chemical composition closely matching that of DNA and quite different from that of proteins. Enzymes such as trypsin and chymotrypsin, known to break down proteins, had no effect on the transforming substance. Ribonuclease, an enzyme that destroys RNA, also had no effect. Enzymes capable of destroying DNA, however, eliminated the biological activity of the transforming substance (**Figure 10.4**). Avery, MacLeod, and McCarty showed that the transforming substance precipitated at about the same rate as purified DNA and that it absorbed ultraviolet light at the same wavelengths as DNA. These results, published in 1944, provided compelling evidence that the transforming principle—and therefore genetic information—resides in DNA. However, new theories in science are rarely accepted on the basis of a single experiment, and many biologists continued to prefer the hypothesis that the genetic material is protein.



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10.4 Avery, MacLeod, and McCarty's experiment revealed the chemical nature of the transforming principle.

Description
The illustration shows a question that reads, What is the chemical nature of the transforming substance? The methods are shown below where a conical flask has Type 3 S (virulent) bacteria. A caption reads, Use heat to kill virulent bacteria, homogenize, and filter. This leads to a flask containing type 3 S bacterial filtrate. A caption reads, Treat samples with enzymes that destroy proteins, R N A, or D N A. This bacterial filtrate is then poured into 3 separate test tubes. RNase (destroys R N A) is added to the first test tube, type 2 R bacteria is obtained in a conical flask. Results below show that type 3 S and type 2 R bacteria is obtained in a conical flask. A caption reads, Cultures treated with protease or RNase contain transformed type 3 S bacteria, but the culture treated with DNase does not. Protease (destroys proteins) is added to the second test tube, type 2 R bacteria is obtained in a conical flask. Results show that type 3 S and type 2 R bacteria is obtained in a conical flask. DNase (destroys D N A) is added to third test tube, type 2 R bacteria is obtained in a conical flask. A caption reads, add the treated samples to cultures of type 2 R bacteria. Results show that type 2 R bacteria is obtained in a conical flask. A caption below reads, Conclusion: Because only DNase destroyed the transforming substance, the transforming substance is D N A.

CONCEPTS

The process of transformation indicates that some substance—the transforming principle—is capable of genetically altering bacteria. Avery, MacLeod, and McCarty demonstrated that the transforming principle is DNA, providing the first evidence that DNA is the genetic material.

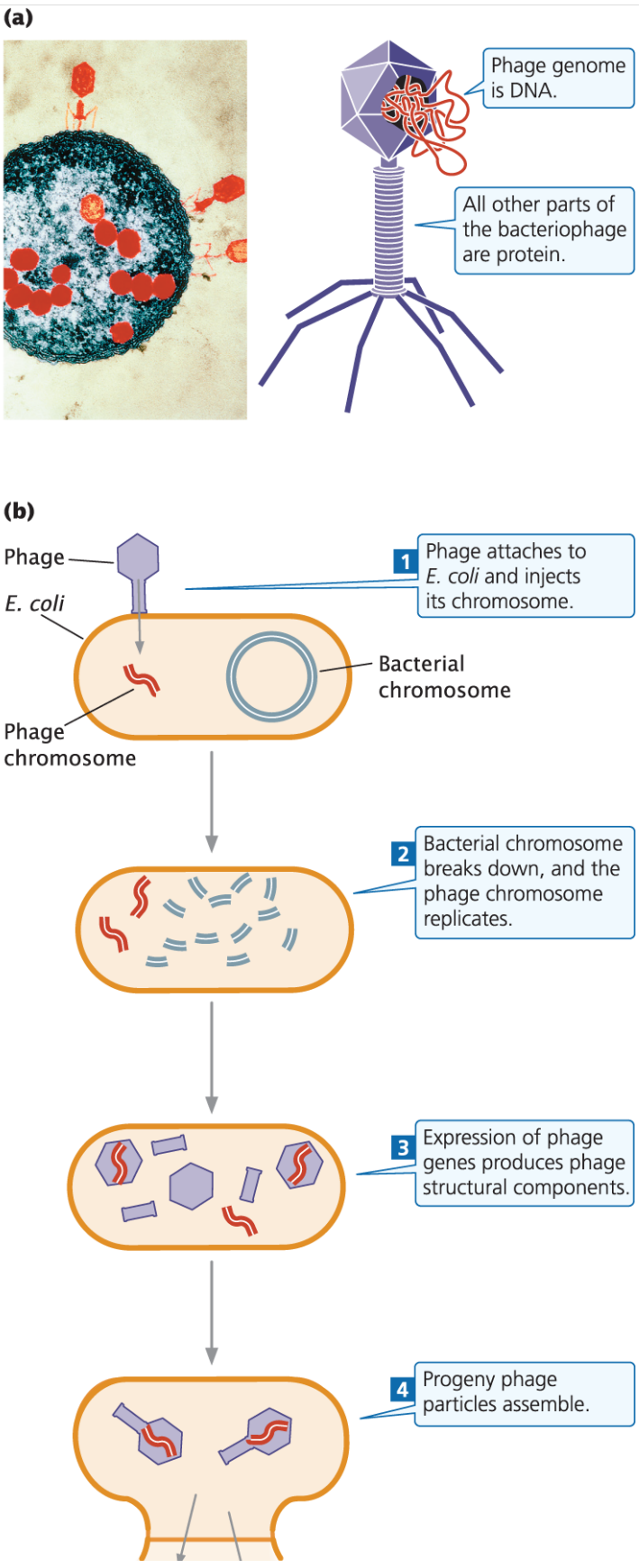
✔ CONCEPT CHECK 3

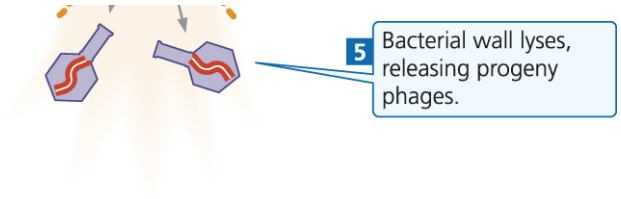
If Avery, MacLeod, and McCarty had found that samples of heat-killed bacteria treated with RNase and DNase transformed bacteria but that samples treated with protease did not, what conclusion would they have drawn?

a. Protease carries out transformation.
b. RNA and DNA are the genetic materials.
c. Protein is the genetic material.
d. RNase and DNase are necessary for transformation.

THE HERSHEY–CHASE EXPERIMENT

A second piece of evidence that indicated DNA was the genetic material resulted from a study of the T2 bacteriophage conducted by Alfred Hershey and Martha Chase. The T2 bacteriophage is a virus that infects the bacterium *Escherichia coli* (**Figure 10.5a**). As we saw in Section 9.5, a phage reproduces by attaching to the outer wall of a bacterial cell and injecting its DNA into the cell, where it replicates and directs the cell to synthesize phage proteins. The phage DNA becomes encapsulated within the phage proteins, producing progeny phages that lyse (break open) the cell and escape (**Figure 10.5b**).


(a)
Phage genome is DNA.
All other parts of the bacteriophage are protein.
(b)
Phage
E. coli
Phage chromosome
1 Phage attaches to E. coli and injects its chromosome.
Bacterial chromosome
2 Bacterial chromosome breaks down, and the phage chromosome replicates.
3 Expression of phage genes produces phage structural components.
4 Progeny phage particles assemble.



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Micrograph: © Lee D. Simon/Science Source.

10.5 T2 is a bacteriophage that infects *E. coli*. (a) T2 phage. (b) Its life cycle.

Description
Illustration a on the left shows multiple T 2 phages over a cell surface along with a diagram that shows structure of a phage. The phage has an icosahedral head with thread-like D N A. A caption reads, Phage genome is D N A. The phage has a tube-shaped body. Another caption reads, All other parts of the bacteriophage are protein. Below the body are 6 legs at its base.

Illustration b on the right shows a virus attaching to an E coli cell with a grey-colored circular chromosome and injecting its red-colored phage chromosome. A caption reads, Phage attaches to E coli and injects its chromosome. This leads to breaking down of the bacterial chromosome and replication of the phage chromosome. A caption reads, Bacterial chromosome breaks down and the phage chromosome replicates. This leads to formation of phage looking structures inside the cell. A caption reads, Expression of phage genes produces phage structural components. This leads to assembling of progeny phages in the cell. A caption reads, Progeny phage particles assemble. This eventually leads to the bacterial wall lyses and release of progeny phages. A caption reads, Bacterial wall lyses, releasing progeny phages.

At the time of the Hershey–Chase study (their paper was published in 1952), biologists did not understand exactly how phages reproduce. What they did know was that the T2 phage is approximately 50% protein and 50% DNA, that a phage infects a bacterial cell by first attaching to the cell wall, and that progeny phages are ultimately produced within the cell. Because the progeny

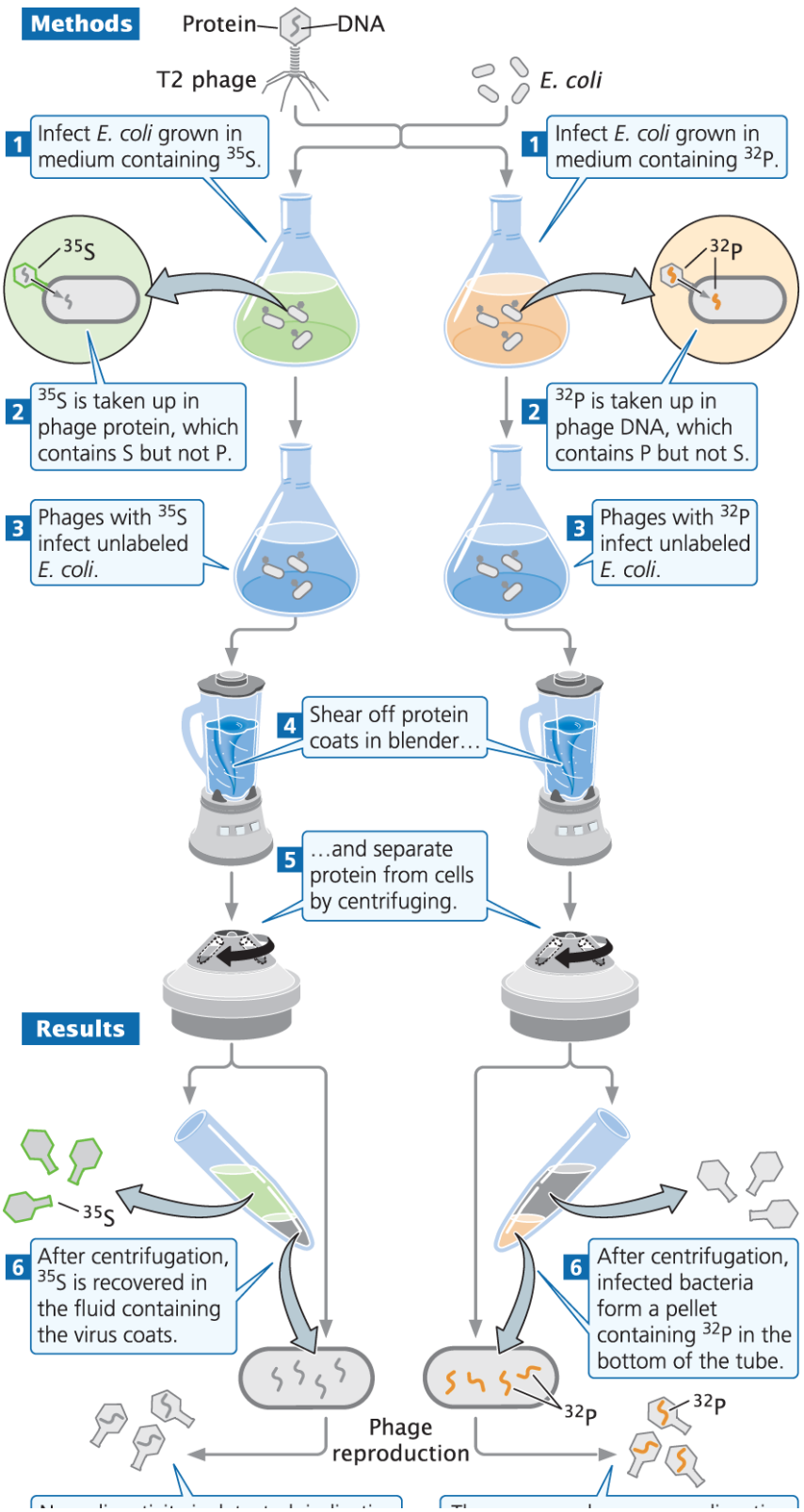
carry the same traits as the infecting phage, genetic material from the infecting phage must be transmitted to the progeny, but how this genetic transmission takes place was unknown.

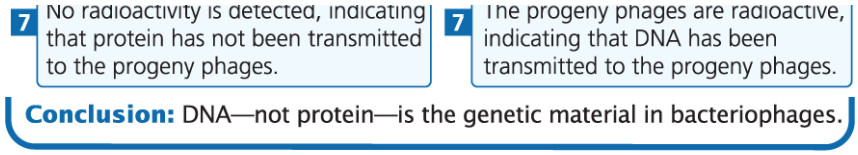
Hershey and Chase designed a series of experiments to determine whether the phage *protein* or the phage *DNA* is transmitted in phage reproduction. To follow the fates of protein and DNA, they used radioactive forms, or **isotopes**, of phosphorus and sulfur. A radioactive isotope can be used as a tracer to identify the location of a specific molecule, because any molecule containing the isotope will be radioactive and therefore easily detected. DNA contains phosphorus but not sulfur, so Hershey and Chase used a radioactive isotope of phosphorus (^{32}P) to follow phage DNA during reproduction. Protein contains sulfur but not phosphorus, so they used a radioactive isotope of sulfur (^{35}S) to follow the protein.

Hershey and Chase grew one batch of *E. coli* in a medium containing ^{32}P and infected the bacteria with the T2 phage so that all the progeny phages would have DNA labeled with ^{32}P (**Figure 10.6**). They grew a second batch of *E. coli* in a medium containing ^{35}S and infected these bacteria with T2 phage so that all the progeny phages would have proteins labeled with ^{35}S. Hershey and Chase then infected separate batches of unlabeled *E. coli* with the ^{35}S- and ^{32}P-labeled progeny phages. After allowing time for the phages to infect the *E. coli* cells, they placed the cells in a blender and sheared off the now-empty phage protein coats from the cell walls. They separated out the protein coats and cultured the infected bacterial cells.

Experiment

Question: Which part of the phage—its DNA or its protein—serves as the genetic material and is transmitted to phage progeny?





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10.6 Hershey and Chase demonstrated that DNA carries the genetic information in bacteriophages.

Description

The illustration begins with the question, Which part of the phage—its D N A or its protein—serves as the genetic material and is transmitted to phage progeny? The methods below show a T 2 phage with its D N A inside the icosahedral head. Two flasks contain E coli and T 2 phage grown in different radioactive materials. A caption pointing to left conical flask reads, Infect E coli in medium containing radioactive sulfur or S left superscript 35. The phage proteins take up the radioactive sulfur. A caption reads, S left superscript 35 is taken up in phage protein, which contains S but not P. On the right side, infect E coli is grown in medium containing P left superscript 32. A caption pointing to the right conical flask reads, Infect E coli grown in medium containing P left superscript 32. Now, the radioactive phosphate is taken up in phage D N A. A caption reads, P left superscript 32 is taken up in phage D N A, which contains P but not S.

On the left side, phages that have S left superscript 35 infect the unlabeled E coli. A caption reads, Phages with S left superscript 35 infect unlabeled E coli. On the right side, phages that have P left superscript 32 infect the unlabeled E coli. A caption reads, Phages with P left superscript 32 infect unlabeled E coli.

The next set of pictures on both left and right show that the labeled phage and unlabeled bacteria are put in a blender to shear off protein coats. Centrifugation is done after the blending. A caption reads, Shear off protein coats in blender and separate protein from cells by centrifugation.

In the results section below, are 2 tubes with pellets and liquid layers. The tube on the left contains radioactive S left superscript 35 in the liquid. An arrow from the bacteria indicates virus coats are radioactive. A caption reads, After centrifugation, S left superscript 35 is recovered in the fluid containing the virus coats. The tube on the right pellet contains radioactive P left superscript 32 bacterial cells. An arrow from the bacteria indicates virus

coat is not radioactive. A caption reads, After centrifugation, infected bacteria form a pellet containing P left superscript 32 in the bottom of the tube.

An arrow pointing down from the left pellet shows a bacterial cell without radiolabeled content. After phage reproduction, progeny phages are obtained. A caption reads, No radioactivity is detected, indicating that protein has not been transmitted to the progeny phages. An arrow pointing down from the right pellet shows a bacterial cell with radiolabeled content. After phage reproduction, progeny phages are obtained. A caption reads, The progeny phages are radioactive, indicating that D N A has been transmitted to progeny phages. A caption below reads, Conclusion: D N A—not protein—is the genetic material in bacteriophages.

In the case of the bacteria infected by phages labeled with ^{35}S, most of the radioactivity was detected in the phage protein coats, and little was detected in the cells. Furthermore, when new phages emerged from the cells, they contained almost no ^{35}S (see Figure 10.6). This result indicated that the protein component of a phage does not enter the cell and is not transmitted to progeny phages.

In contrast, when Hershey and Chase infected bacteria with ^{32}P-labeled phages and then removed the phage protein coats, the bacteria were radioactive. Most significantly, after the bacterial cells were lysed and new progeny phages emerged, many of those phages emitted radioactivity, demonstrating that DNA from the infecting phages had been passed on to the progeny phages (see Figure 10.6). These results confirmed that DNA, not protein, is the genetic material of phages. › **TRY PROBLEM 24**

CONCEPTS

Using radioactive isotopes, Hershey and Chase traced the movement of DNA and protein during phage infection of bacteria. They demonstrated that DNA, not protein, enters the bacterial cell during phage reproduction and that only DNA is passed on to progeny phages.

✔ CONCEPT CHECK 4

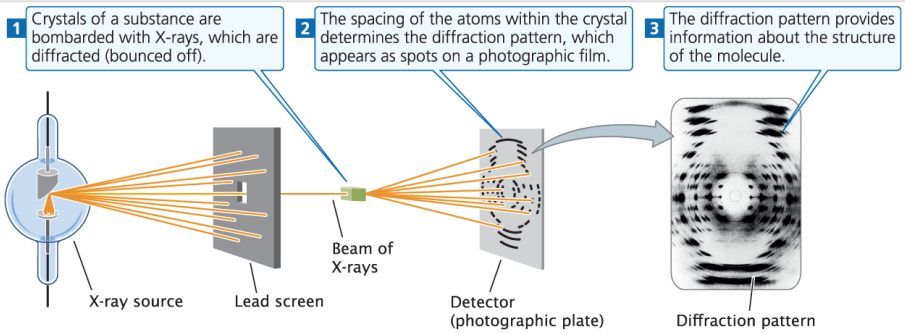
Could Hershey and Chase have used a radioactive isotope of carbon instead of ^{32}P? Why or why not?

Watson and Crick's Discovery of the Three-Dimensional Structure of DNA

These experiments on the nature of the genetic material set the stage for one of the most important advances in the history of biology: the discovery of the three-dimensional structure of DNA by James Watson and Francis Crick in 1953.

Before Watson and Crick's breakthrough, much of the basic chemistry of DNA had already been determined by Miescher, Kossel, Levene, Chargaff, and others, who had established that DNA consists of nucleotides and that each nucleotide contains a sugar, a nitrogenous base, and a phosphate group. However, how the nucleotides fit together in the three-dimensional structure of the molecule was not at all clear.

In 1947, William Astbury began studying the three-dimensional structure of DNA by using a technique called **X-ray diffraction** (**Figure 10.7**), in which X-rays beamed at a molecule are reflected in specific patterns that reveal aspects of the structure of the molecule. However, his diffraction images did not provide enough resolution to reveal the structure. A research group at King's College in London, led by Maurice Wilkins, also used X-ray diffraction to study DNA. Working in Wilkins's laboratory, Rosalind Franklin obtained strikingly better images of the molecule. Rosalind Franklin was an excellent X-ray crystallographer, and her images were critical in the eventual elucidation of the structure of DNA by Watson and Crick.



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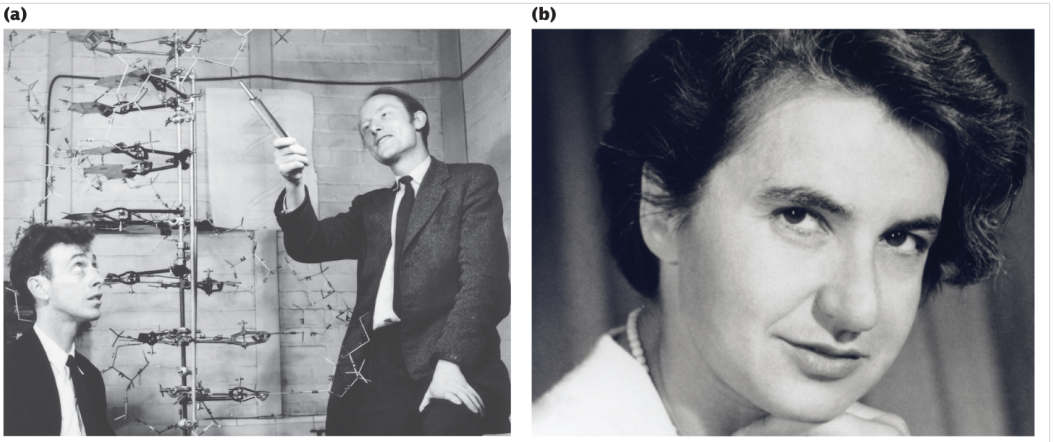
10.7 X-ray diffraction provides information about the structure of molecules.

Description

The illustration shows a circular tube placed on the left. The tube is the source of X-rays that scatter an array of rays towards the surface of the flat lead screen placed on the right. A square hole in the middle of the lead screen allows a beam of X-ray to pass through. This beam hits a square substance and is scattered. A caption reads, Crystals of a substance are bombarded with X-rays, which are diffracted or bounce off. The scattered X-rays then fall on a detector (photographic plate). A caption reads, The spacing of the atoms within the crystal determines the diffraction pattern, which appears as spots on a

photographic film. Finally, a diffraction pattern is obtained on the right side. A caption reads, The diffraction pattern provides information about the structure of the molecule.

Watson and Crick investigated the structure of DNA not by collecting new data but by using all available information about the chemistry of DNA to construct molecular models (Figure 10.8a). By using the excellent X-ray diffraction images taken by Rosalind Franklin (Figure 10.8b) and by applying the laws of structural chemistry, they were able to limit the number of possible structures that DNA could assume. They tested various structures by building models made of wire and metal plates. With their models, they were able to see whether a structure was compatible with chemical principles and with the X-ray images.



Part a: A. Barrington/Science Photo Library/Science Source. Part b: National Library of Medicine/Science Source.

10.8 James Watson and Francis Crick (a) developed a three-dimensional model of the structure of DNA based in part on X-ray diffraction images taken by Rosalind Franklin (b).

The key to solving the structure came when Watson recognized that an adenine base could bond with a thymine base and that a guanine base could bond with a cytosine base; these pairings accounted for the base ratios that Chargaff had discovered earlier. The model developed by Watson and Crick showed that DNA consists of two strands of nucleotides that run in opposite directions (are antiparallel) and wind around each other to form a right-handed helix, with the sugars and phosphates on the outside and the bases in the interior. They recognized that the double-stranded structure of DNA, with its specific base pairing, provided an elegant means by which DNA could be replicated. Watson and Crick published an electrifying description of their model in *Nature* in 1953. At the same time, Franklin and Wilkins each published their X-ray diffraction data, which demonstrated that DNA was helical in structure.

Many have called the solving of DNA's structure the most important biological discovery of the twentieth century. For their discovery, Watson and Crick, along with Maurice Wilkins, were awarded the Nobel Prize in chemistry in 1962. Rosalind Franklin had died of cancer in 1958 and thus could not be considered a candidate for the shared prize, but many scholars and historians believe that she should receive equal credit for solving the structure of DNA.

Following the discovery of DNA's structure, much research was focused on how genetic information is encoded within the base sequence and how this information is copied and expressed. Even

today, the details of DNA structure and function continue to be the subject of active research.

THINK-PAIR-SHARE Questions 2 and 3

CONCEPTS

Using data collected by Rosalind Franklin, Maurice Wilkins, and others, and building molecular models, Watson and Crick were able to discover the three-dimensional structure of the DNA molecule.

✔ CONCEPT CHECK 5

What did Watson and Crick use to help solve the structure of DNA?

a. X-ray diffraction images
b. Laws of structural chemistry
c. Models of DNA
d. All of the above

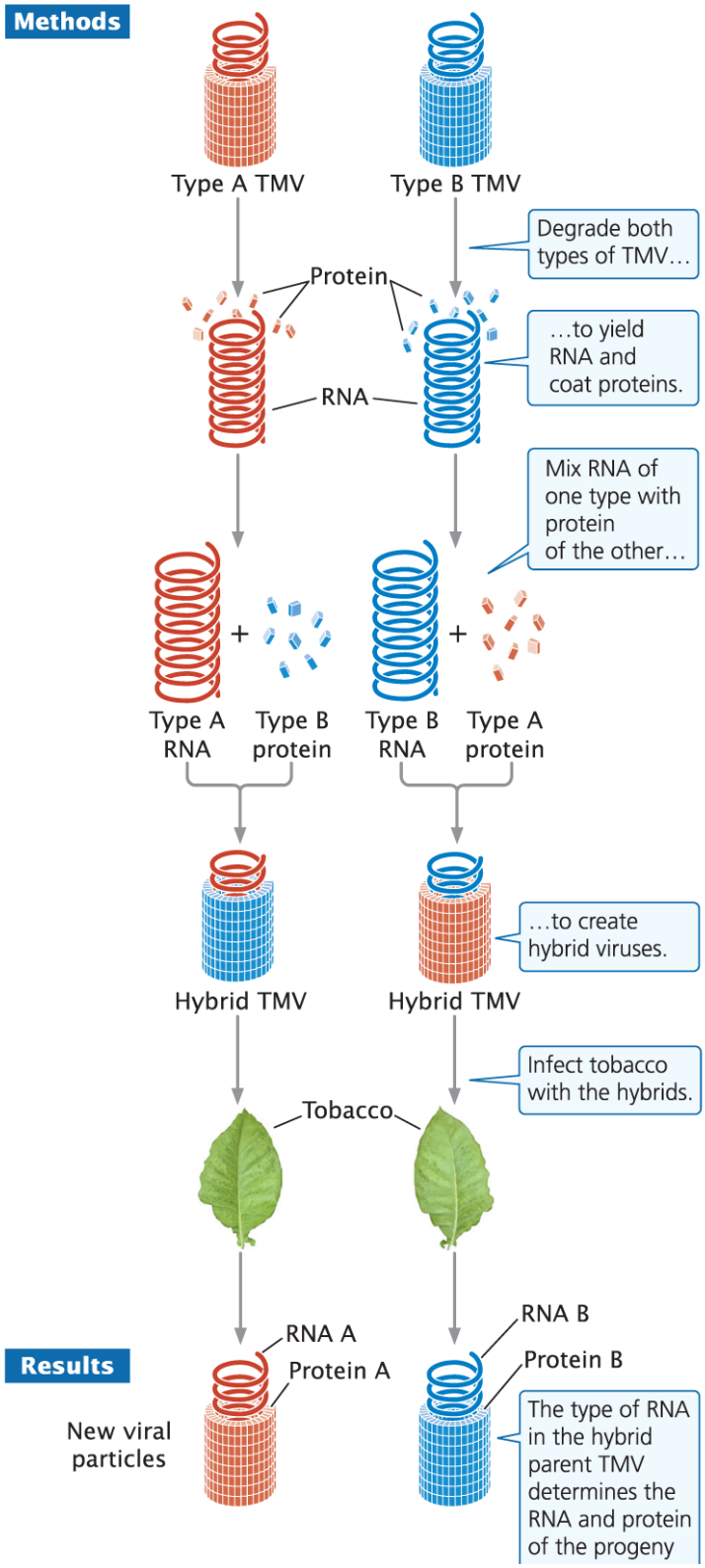
RNA as Genetic Material

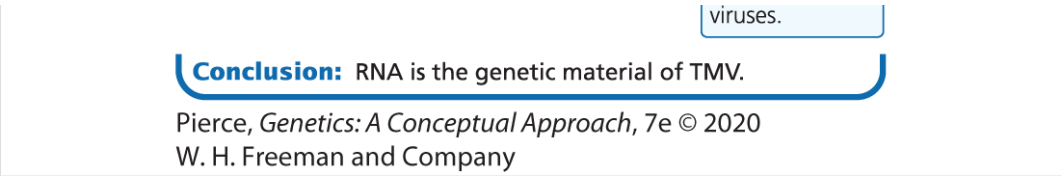
In most organisms, DNA carries the genetic information. A few viruses, however, use RNA, not DNA, as their genetic material. This was demonstrated in 1956 by Heinz Fraenkel-Conrat and Bea Singer, who worked with the tobacco mosaic virus (TMV), which infects and causes disease in tobacco plants (Figure 10.9). The tobacco mosaic

virus possesses a single molecule of RNA surrounded by a helically arranged cylinder of protein molecules. Fraenkel-Conrat found that after separating the RNA and the protein of TMV, he could remix the RNA and protein of different strains of TMV and obtain intact, infectious viral particles.

Experiment

Question: What substance—RNA or protein—carries the genetic material in tobacco mosaic virus (TMV)?





10.9 Fraenkel-Conrat and Singer's experiment demonstrated that RNA carries the genetic information in tobacco mosaic virus.

Description

The illustration shows the question at the top which reads, What substance—R N A or protein—carries the genetic material in tobacco mosaic virus (T M V)? The methods section below shows a red tobacco mosaic virus labeled as T M V type A on the left and a blue tobacco mosaic virus labeled as T M V type B on the right. The virus is cylindrical and its R N A is shaped like a coiled spring. The viruses are degraded to obtain coat proteins and their respective R N A. A caption reads, Degrade both types of T M V to yield R N A and coat proteins. Red type A-R N A is now mixed with type B protein and blue type B-R N A is mixed with type A protein. A caption reads, Mix R N A of one type with protein of the other to create hybrid viruses. The blue virus has a red R N A inside and the red virus has a blue R N A inside. Both viruses are labeled as hybrid T M V. Two tobacco leaves are infected with one each of these hybrid T M V's. A caption reads, Infect tobacco with the hybrids.

The results below show new viral particles. The tobacco leaf infected with hybrid T M V that has blue protein and red R N A eventually produces a virus with red protein and red R N A type A. The tobacco leaf infected with hybrid T M V with red protein and blue R N A eventually produces a virus with blue protein and blue R N A type B. A caption reads, The type of R N A in the hybrid parent T M V determines the R N A and protein of the progeny viruses. A caption below reads, Conclusion: RNA is the genetic material of T M V.

With Singer, Fraenkel-Conrat then created hybrid viruses by mixing RNA and protein from different strains of TMV. When these hybrid viruses infected tobacco leaves, new viral particles were produced. The new viral progeny were identical to the strain from which the RNA had been isolated and did not exhibit the characteristics of the

strain that donated the protein. These results showed that RNA carries the genetic information in TMV.

Also in 1956, Alfred Gierer and Gerhard Schramm demonstrated that RNA isolated from TMV is sufficient to infect tobacco plants and direct the production of new TMV particles. This finding confirmed that RNA carries the genetic instructions in this virus. › **TRY PROBLEM 18**

CONCEPTS

RNA serves as the genetic material in some viruses.

10.3 DNA Consists of Two Complementary and Antiparallel Nucleotide Strands That Form a Double Helix

DNA, though relatively simple in structure, has an elegance and beauty unsurpassed by other large molecules. It is useful to consider the structure of DNA at three levels of increasing complexity, known as the primary, secondary, and tertiary structures of DNA. The primary structure of DNA refers to its nucleotide structure and how the nucleotides are joined together. The secondary structure refers to DNA's stable three-dimensional configuration, the helical structure worked out by Watson and Crick. In Chapter 11, we will consider DNA's tertiary structures, which are the complex packing arrangements of double-stranded DNA in chromosomes.

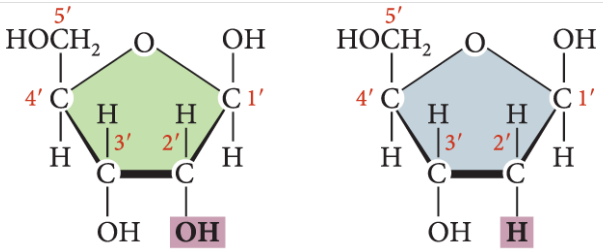
The Primary Structure of DNA

The primary structure of DNA consists of a string of nucleotides joined together by phosphodiester linkages.

NUCLEOTIDES

DNA is typically a very long molecule and is therefore termed a macromolecule. For example, the DNA in each human chromosome is a single molecule that, if stretched out straight, would be several centimeters in length, thousands of times longer than a human cell. In spite of its large size, DNA has quite a simple structure: it is a polymer—that is, a chain made up of many repeating units linked together. The repeating units of DNA are nucleotides, each comprising three parts: (1) a sugar, (2) a phosphate group, and (3) a nitrogen-containing base.

The sugars of nucleic acids—called pentose sugars—have five carbon atoms, numbered $1'$, $2'$, $3'$, $4'$, and $5'$ (**Figure 10.10**). The sugars of DNA and RNA are slightly different in structure. RNA's sugar, called **ribose**, has a hydroxyl group (OH) attached to the $2'$-carbon atom, whereas DNA's sugar, or **deoxyribose**, has a hydrogen atom (H) at this position and therefore contains one oxygen atom fewer overall. This difference gives rise to the names ribonucleic acid (RNA) and *deoxy*ribonucleic acid (DNA). This minor chemical difference is recognized by most of the cellular enzymes that interact with DNA or RNA, thus providing specific functions for each nucleic acid. Furthermore, the additional oxygen atom in the RNA nucleotide makes it more reactive and less chemically stable than DNA. For this reason, DNA is better suited to serve as the long-term carrier of genetic information.



Ribose **Deoxyribose**

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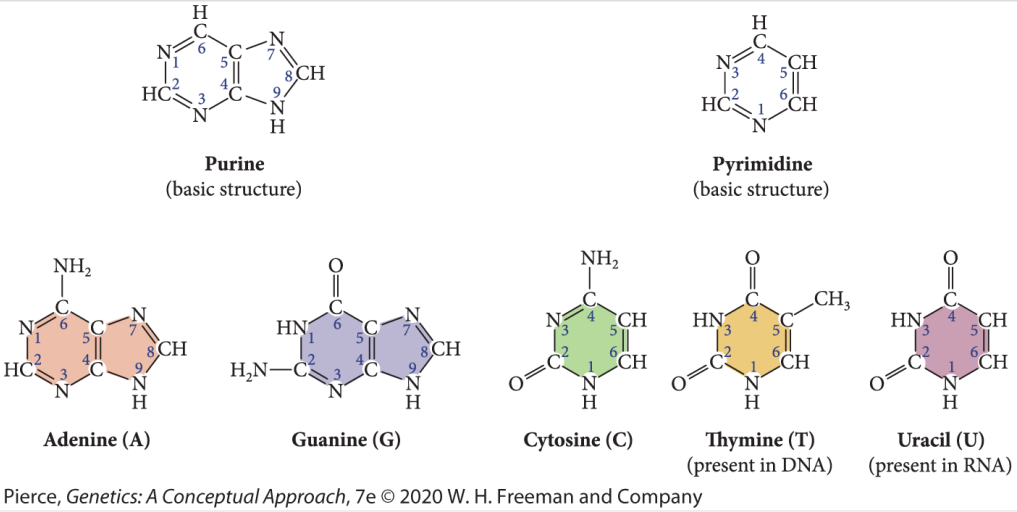
10.10 A nucleotide contains either a ribose sugar (in RNA) or a deoxyribose sugar (in DNA). The carbon atoms of the sugars are assigned primed numbers.

Description

The illustration shows both the sugar molecules have pentagonal rings. The top of the ring is occupied by an O and the other vertices of the pentagon have C atoms. The carbon on the right is designated as 1 prime, and moving clockwise, they are labeled as 2, 3, and 4 prime. For ribose sugar, the carbon atoms 1 through 3 have one hydroxyl group and one hydrogen atom each. For deoxyribose, carbon 2 prime has both hydrogen atoms instead. For both ribose and deoxyribose sugars, 4 prime carbon is bound to one hydrogen atom and to a C atom labeled as 5 prime. The 5 prime C atom has 2 hydrogen bonds and one hydroxyl bond.

The second component of a nucleotide is its **nitrogenous base**, which may be either of two types—a **purine** or a **pyrimidine** (**Figure 10.11**). Each purine consists of a six-member ring attached to a five-member ring, whereas each pyrimidine consists of a six-member ring only. Both DNA and RNA contain two purines, **adenine** and **guanine** (A and G), which differ in the positions of their double bonds and in the groups attached to the six-member ring. Three pyrimidines are common in nucleic acids: **cytosine** (C), **thymine** (T), and **uracil** (U). Cytosine is present in both DNA and RNA; however,

thymine is restricted to DNA, and uracil is found only in RNA. The three pyrimidines differ in the groups or atoms attached to the carbon atoms of the ring and in the number of double bonds in the ring. In a nucleotide, the nitrogenous base always forms a covalent bond with the 1′-carbon atom of the sugar (see Figure 10.10). A deoxyribose or a ribose sugar and a base together are referred to as a **nucleoside**.



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10.11 A nucleotide contains either a purine or a pyrimidine base. The atoms of the rings in the bases are assigned unprimed numbers.

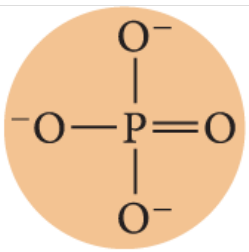
Description

The structure of purine contains a pentagon bound to a hexagon. The numbering of the rings starts with one of the nitrogens on the hexagon. The atoms from positions 2 to 6 are C 2, N 3, C 4, C 5, and C 6 which is double bonded to N 1. Every alternate bond in the hexagonal ring is a double bond. In other words, there is a double bond between positions 2 and 3, 4 and 5, and 6 and 1. The C 4 C 5 double bond of the hexagon is shared with the pentagon. Positions 7 through 9 are in the pentagon. N 7 is bonded to the C 5 carbon. The next 2 atoms are C 8 and N 9, which is then bonded with the hexagon at position C 4. Between N 7 and C 8 is another double bond. C 2, C 6, C 8, and N 9 are each bonded to a hydrogen atom. Below this purine structure, are the structures of bases, adenine and

guanine. The adenine structure is identical to that of the purine, with the exception that the hydrogen at C 6 is replaced by an amine group (N H subscript 2). The structure of guanine is identical to that of the purine, with the exception that the hydrogen at C 2 is replaced by an amine group (N H subscript 2).

On the right side, is the basic structure of pyrimidine. It has the shape of a hexagon and is almost identical to the structure of the hexagonal ring in the purine. The only difference between the two is the orientation of the double bonds, which are drawn in the opposite orientation. In other words, between positions 1 and 2, 3 and 4, and 5 and 6. Below the basic structure of pyrimidine, are the structures of cytosine, thymine, and uracil. The structure of cytosine is identical to the basic pyrimidine, except there are differences at carbons 2 and 4. The double bond between C 2 and N 1 is absent, the hydrogen at C 2 is replaced with a double bond to an oxygen atom. The hydrogen at the C 4 of cytosine is replaced with an amine group (N H subscript 2). Next is the structure of thymine, which is only present in D N A. Thymine is identical to cytosine, except at carbons 4 and 5. C 4 is no longer double bonded with N 3 and now has a double bond with an oxygen atom. C 5's hydrogen atom is replaced with a methyl group (C H subscript 3). Next is the structure of uracil, which is only found in R N A. The structure of uracil is identical with that of thymine, except that the methyl group on carbon 5 is replaced with a hydrogen atom.

The third component of a nucleotide is its **phosphate group**, which consists of a phosphorus atom bonded to four oxygen atoms (**Figure 10.12**). Phosphate groups are found in every nucleotide and frequently carry a negative charge, which makes DNA acidic. The phosphate group is always bonded to the 5′-carbon atom of the sugar (see Figure 10.10) in a nucleotide.

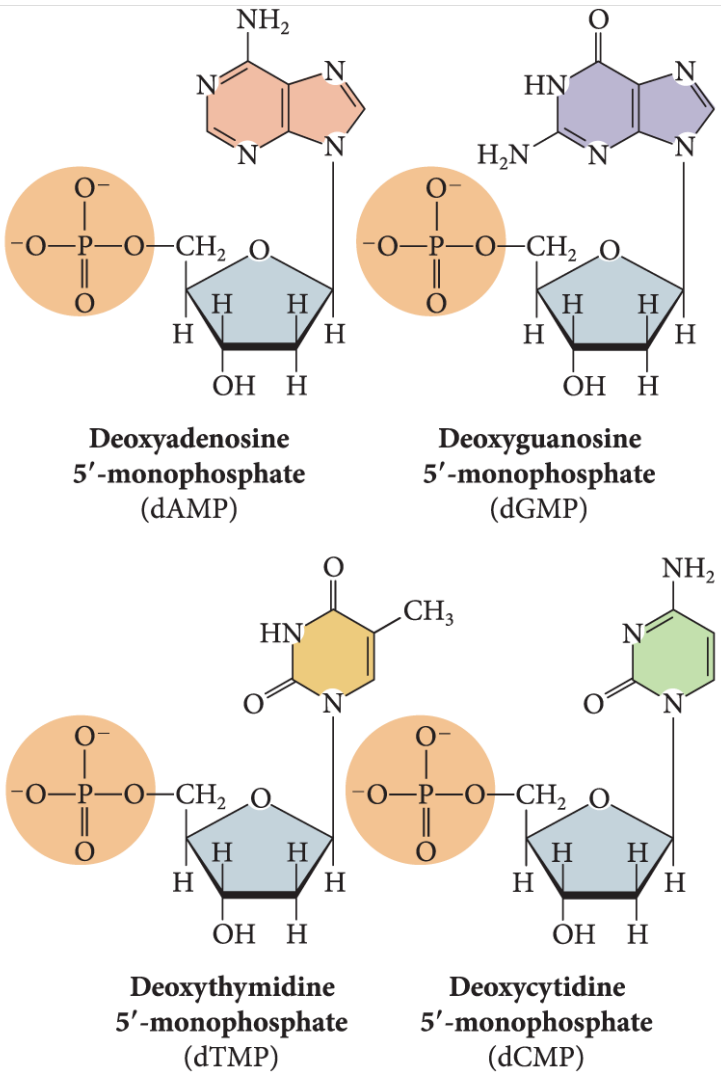


Phosphate

10.12 A nucleotide contains a phosphate group.

Description
The phosphate group has one central P atom bonded to 4 oxygen atoms at right angles. One of the oxygen atoms is double bonded, and the remaining three are single-bonded and have single negative charges associated with them.

The DNA nucleotides are properly known as **deoxyribonucleotides**, or deoxyribonucleoside 5′-monophosphates. Because there are four types of bases, there are four different kinds of DNA nucleotides (**Figure 10.13**). The equivalent RNA nucleotides are termed **ribonucleotides**, or ribonucleoside 5′-monophosphates. RNA molecules sometimes contain additional rare bases, which are modified forms of the four common bases. These modified bases will be discussed in more detail when we examine the function of RNA molecules in Chapter 14. The names for DNA bases, nucleotides, and nucleosides are shown in **Table 10.2**. **› TRY PROBLEM 26**



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10.13 There are four types of DNA nucleotides. These nucleotides are deoxyribonucleoside 5′-monophosphates.

Description

The phosphate group at the top has one central P atom bonded to 4 oxygen atoms at right angles. One of the oxygen atoms is double bonded, and the remaining three are single-bonded and have single negative charges associated with them.

The illustration also shows four types of D N A nucleotides below. Each of the four D N A nucleotides is drawn as a central deoxyribose sugar with a single phosphate group attached to the 5 prime carbon and with the base pair on top attached through the C 1

prime carbon. For Deoxyadenosine 5 prime monophosphate or d A M P shown at top left, the hydroxyl group at C 1 of the deoxyribose is removed and is bound to the adenine structure through the N 9 position. Deoxyguanosine 5 prime monophosphate or d G M P shown at top right, also has the hydroxyl group at C 1 removed and is bound to guanine through the N 9 position. Deoxythymidine 5 prime monophosphate or d T M P shown at bottom left, has the thymine base pair attached to the C 1 through the N 1 position of the thymine. Deoxycytidine 5 prime monophosphate or d C M P shown at bottom right, is also attached to the C 1 position of the deoxyribose through the N 1 of the cytosine.

TABLE 10.2 Names of DNA bases, nucleotides, and nucleosides

	Adenine	Guanine	Thymine	Cytosine
Base symbol	A	G	T	C
Nucleotide	deoxyadenosine 5′-monophosphate	deoxyguanosine 5′-monophosphate	deoxythymidine 5′-monophosphate	deoxycytidine 5′-monophosphate
Nucleotide symbol	dAMP	dGMP	dTMP	dCMP
Nucleoside	deoxyadenosine	deoxyguanosine	deoxythymidine	deoxycytidine
Nucleoside symbol	dA	dG	dT	dC

CONCEPTS

The primary structure of DNA consists of a string of nucleotides. Each nucleotide consists of a five-carbon sugar, a phosphate group, and a nitrogenous base. There are two types of DNA bases: purines (adenine and guanine) and pyrimidines (thymine and cytosine).

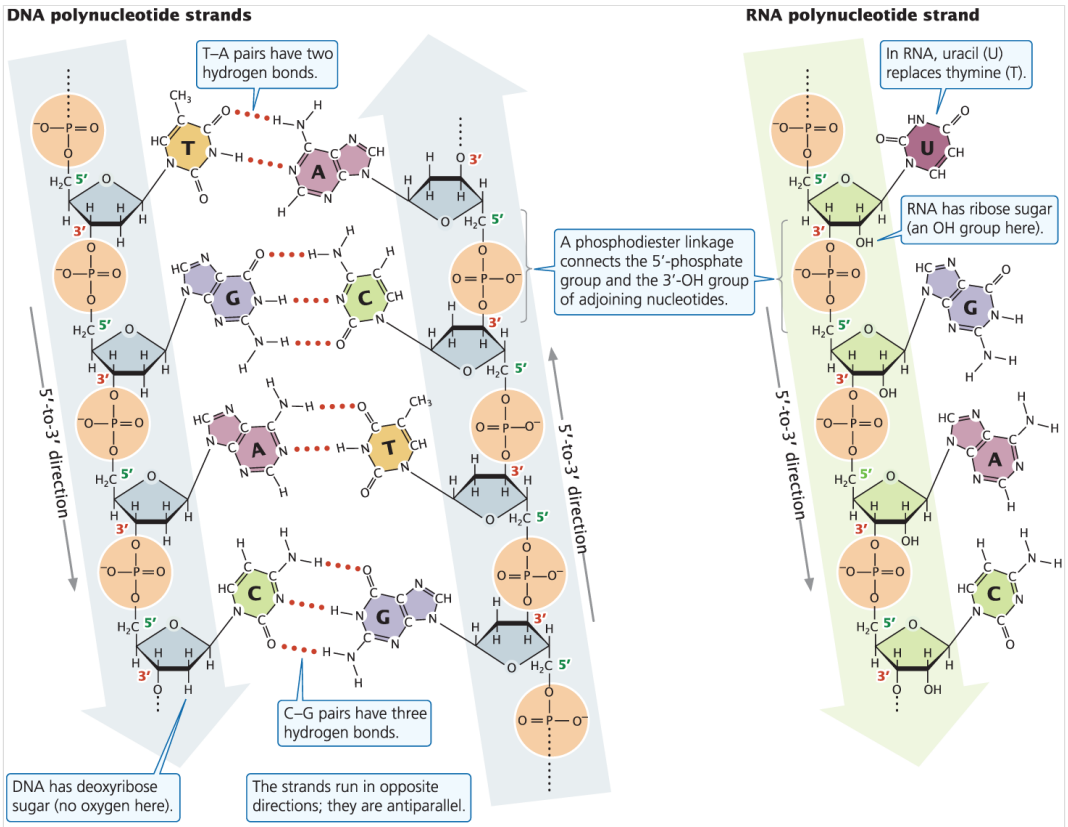
✔ CONCEPT CHECK 6

How do the sugars of RNA and DNA differ?

a. RNA has a six-carbon sugar; DNA has a five-carbon sugar.
b. The sugar of RNA has a hydroxyl group that is not found in the sugar of DNA.
c. RNA contains uracil; DNA contains thymine.
d. DNA's sugar has a phosphorus atom; RNA's sugar does not.

POLYNUCLEOTIDE STRANDS

DNA is made up of many nucleotides connected by covalent bonds, which join the 5′-phosphate group of one nucleotide to the 3′-hydroxyl group of the next nucleotide (**Figure 10.14**). The structures shown in Figure 10.14 are flattened into two dimensions, although the molecule itself is three-dimensional, as shown in Figure 10.15a. Note that Figure 10.14 shows only a portion of the DNA and RNA molecules; each strand continues at its 5′ and 3′ ends, as indicated by ••••. The bonds that connect nucleotides, called **phosphodiester linkages**, are strong covalent bonds; a series of nucleotides linked in this way constitutes a **polynucleotide strand**. The backbone of the polynucleotide strand is composed of alternating sugars and phosphate groups; the bases project away from the long axis of the strand. The negative charges of the phosphate groups are frequently neutralized by their association with positive charges on proteins, metals, or other molecules.



10.14 DNA and RNA are composed of polynucleotide strands. DNA is usually composed of two polynucleotide strands, although single-stranded DNA is found in some viruses. This figure shows only a portion of larger DNA and RNA molecules; each strand continues at its 5′ and 3′ ends.

Description

The D N A strand on the left has nucleotides T, G, A, and C in a chain (from 5 prime at top to 3 prime at bottom) with the A, C, T, and G pairing respectively with them on the opposite strand (from 3 prime at top to 5 prime at bottom). In the sugar molecule, the C 3 prime atom's hydroxyl groups are replaced with the next nucleotide in the chain, joined through one of the oxygen atoms in the phosphate group. A caption reads, T-A pairs have 2 hydrogen bonds and C-G pairs have 3 hydrogen bonds. The double bonded oxygen, N 3, and a hydrogen atom from the amine group of deoxycytosine interacts with the hydrogen, N atom and oxygen atoms from deoxyguanosine. The N 2 and hydrogen atom from the amine group of deoxyadenosine, interact with the double bonded oxygen and hydrogen atom from deoxythymidine. A caption reads, The strands run in opposite directions; they are antiparallel. Another caption reads, A phosphodiester linkage connects the 5 prime

phosphate group and the 3 prime O H group of adjoining nucleotides. Another caption reads, D N A has a deoxyribose sugar, which has no oxygen on the C 2 atom. The illustration also shows an R N A strand on the right from 5 prime (top) to 3 prime (bottom) in which nucleotides U, G, A, and C are present. In the sugar molecule, the C 3 prime atom's hydroxyl groups are replaced with the next nucleotide in the chain, joined through one of the oxygen atoms in the phosphate group. A caption reads, In R N A, uracil (U) replaces the thymine (T). R N A has ribose sugar and has one O H group on the C 2 atom. A caption reads, R N A has ribose sugar (an O H group here).

An important characteristic of the polynucleotide strand is its direction, or polarity. At one end of the strand, a free phosphate group (unattached on one side) is attached to the 5′-carbon atom of the sugar in the nucleotide. This end of the strand is therefore referred to as the **5′ end**. The other end of the strand, referred to as the **3′ end**, has a free hydroxyl group attached to the 3′-carbon atom of the sugar. RNA nucleotides are also connected by phosphodiester linkages to form similar polynucleotide strands (see Figure 10.14).

CONCEPTS

The nucleotides of DNA are joined into polynucleotide strands by phosphodiester bonds that connect the 3′-carbon atom of one nucleotide to the 5′-phosphate group of the next. Each polynucleotide strand has polarity, with a 5′ end and a 3′ end.

Secondary Structures of DNA

The secondary structure of DNA refers to its three-dimensional configuration—its fundamental helical structure. DNA's secondary structure can assume a variety of configurations, depending on its base sequence and the conditions in which it is placed.

THE DOUBLE HELIX

A fundamental characteristic of DNA's secondary structure is that it consists of two polynucleotide strands wound around each other—it's a *double* helix. The sugar–phosphate linkages are on the outside of the helix, and the bases are stacked in the interior of the molecule (see Figure 10.14). The two polynucleotide strands run in opposite directions—they are **antiparallel**, which means that the 5′ end of one strand is opposite the 3′ end of the other strand.

The two strands are held together by two types of molecular forces. Hydrogen bonds link the bases on opposite strands (see Figure 10.14). These bonds are relatively weak compared with the covalent phosphodiester bonds that connect the sugars and phosphate groups of adjoining nucleotides on the same strand. As we will see, several important functions of DNA require the separation of its two nucleotide strands, and this separation can be readily accomplished because of the relative ease of breaking and reestablishing the hydrogen bonds.

The nature of the hydrogen bond imposes a limitation on the types of bases that can pair. Adenine normally pairs only with thymine through two hydrogen bonds, and cytosine normally pairs only with guanine through three hydrogen bonds (see Figure 10.14). Because three hydrogen bonds form between C and G and only two hydrogen bonds form between A and T, C–G pairing is stronger than A–T pairing. The specificity of the base pairing means that wherever there is an A on one strand, there must be a T in the corresponding position on the other strand, and wherever there is a G on one strand, a C must be on the other. The two polynucleotide strands of a DNA molecule are therefore not identical but rather **complementary DNA strands**. The complementary nature of the two nucleotide strands provides for efficient and accurate DNA replication, as we will see in Chapter 12.

The second force that holds the two DNA strands together is the interaction between the stacked base pairs in the interior of the molecule. Stacking means that adjacent bases are aligned so that their rings are parallel and stack on top of one another. The stacking interactions stabilize the DNA molecule but do not require that any particular base follow another. Thus, the base sequence of the DNA molecule is free to vary, allowing DNA to carry genetic information.

› TRY PROBLEMS 32 AND 38

THINK-PAIR-SHARE QUESTION 4

CONCEPTS

DNA consists of two polynucleotide strands. The sugars and phosphate groups of each polynucleotide strand are on the outside of the molecule, and the bases are in the interior. Hydrogen bonding joins the bases of the two strands: guanine pairs with cytosine, and adenine pairs with thymine. The two polynucleotide strands of a DNA molecule are complementary and antiparallel.

✔ CONCEPT CHECK 7

The antiparallel nature of DNA refers to

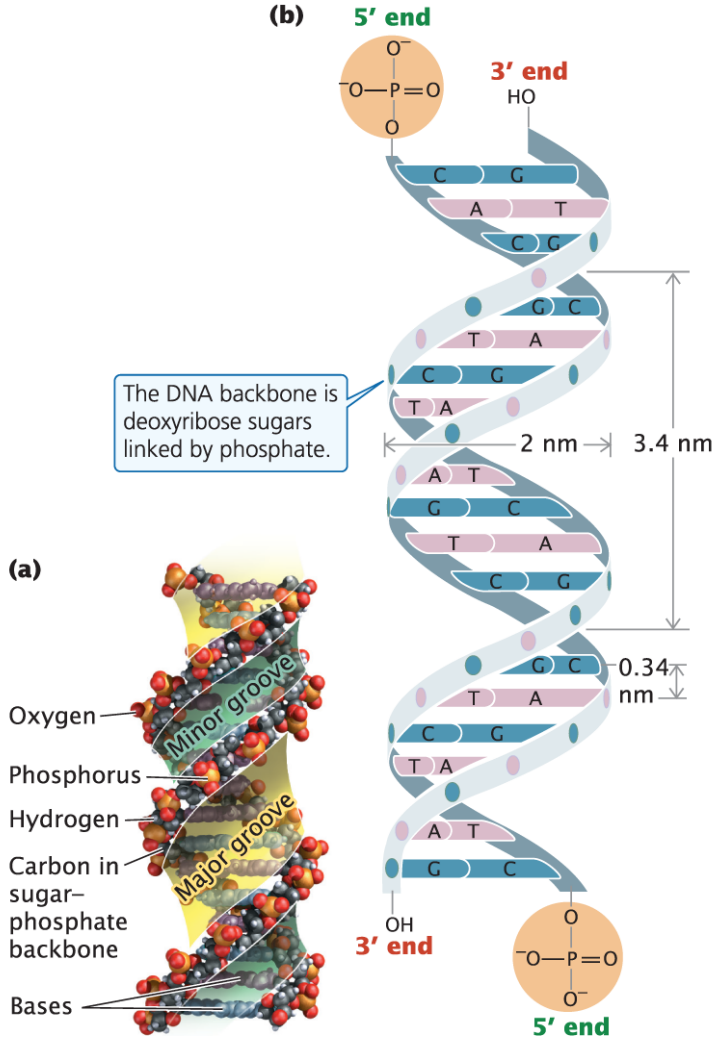
a. its charged phosphate groups.
b. the pairing of bases on one strand with bases on the other strand.
c. the formation of hydrogen bonds between bases from opposite strands.
d. the opposite direction of the two strands of nucleotides.

DIFFERENT SECONDARY STRUCTURES

As we have seen, DNA normally consists of two polynucleotide strands that are antiparallel and complementary (exceptions are the single-stranded DNA molecules found in a few viruses). The precise three-dimensional shape of the molecule can vary, however, depending on the conditions in which the DNA is placed and, in some cases, on the base sequence itself.

The three-dimensional structure of DNA described by Watson and Crick is termed the **B-DNA** structure (**Figure 10.15**). This structure exists when plenty of water surrounds the molecule and there are no

unusual base sequences in the DNA—conditions that are likely to be present in cells. The B-DNA structure is the most stable configuration for a random sequence of nucleotides under physiological conditions, and most evidence suggests that it is the predominant structure in the cell.



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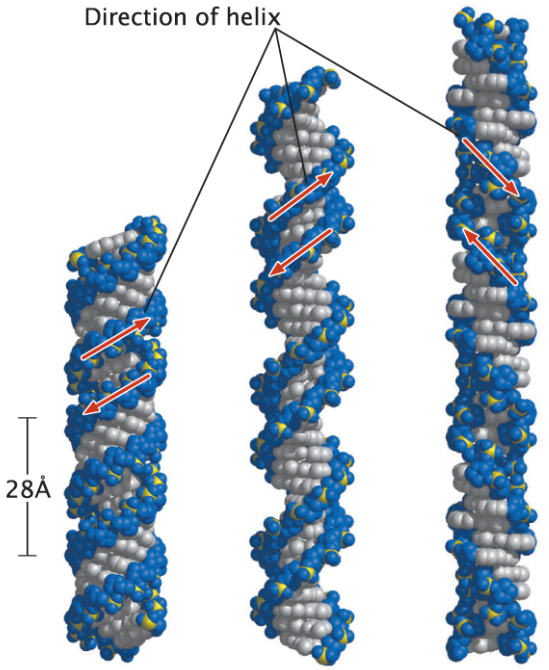
10.15 B-DNA consists of a right-handed helix with approximately 10 bases per turn. (a) Space-filling model of B-DNA showing major and minor grooves. (b) Diagrammatic representation.

Description
Illustration a, the space model on the left, has several round circles of different colors labeled as oxygen, phosphorus, hydrogen, and carbon in sugar-phosphate backbone. Base pairs are also labeled. The major groove is labeled where the backbones are far apart in the helix and the minor groove is marked where they are close together. Illustration b on the right shows one strand of the helix with a phosphate group and is labeled as the 5 prime end, and on the opposing strand an O H group is present and is labeled the 3 prime end. A caption reads, The D N A backbone has deoxyribose sugars linked by phosphate. The illustrations also show the dimensions of double helix. The width is 2 nanometers. From minor groove to minor groove, the distance is 3 point 4 nanometers. From base pair to base pair, the distance is 0 point 3 4 nanometers.

B-DNA is a right-handed helix, meaning that it has a clockwise spiral. There are approximately 10 base pairs (bp) per 360-degree rotation of the helix, so each base pair is twisted 36 degrees relative to the adjacent bases (see **Figure 10.15b**). The base pairs are 0.34 nanometers (nm) apart, so each complete rotation of the molecule encompasses 3.4 nm. The diameter of the helix is 2 nm, and the bases are perpendicular to the long axis of the DNA molecule. A space-filling model shows that B-DNA has a slim and elongated structure (see **Figure 10.15a**). The spiraling of the nucleotide strands creates major and minor grooves in the helix, features that are important for the binding of some proteins that regulate the expression of genetic information (see Chapter 16).

Another secondary structure that DNA can assume is the **A-DNA** structure, which exists if less water is present. Like B-DNA, A-DNA is a right-handed helix (**Figure 10.16a**), but it is shorter and wider than B-DNA (**Figure 10.16b**), and its bases are tilted away from the main

axis of the molecule. A-DNA has been detected in some DNA–protein complexes and in spores of some bacteria.



After J. M. Berg, J. L. Tymoczko, and L. Stryer, *Biochemistry*, 6th ed. (New York: W. H. Freeman and Company, 2002), pp. 785 and 787.

10.16 DNA can assume several different secondary structures.

Description

Illustration a on the left shows A form which is relatively smaller than the B form and measures 28 angstroms from minor groove to minor groove. The direction of first groove is upwards at almost an angle of 30 degrees and the second groove is downwards at an angle of almost 30 degrees. Illustration b in the center shows B form which is a little longer than the A form and its grooves are oriented in the same direction as A form. Illustration c on the right shows Z form which is relatively longer than the B form and the grooves are oriented in opposite direction.

A radically different secondary structure, called Z-DNA (Figure 10.16c), forms a left-handed helix. In this structure, the sugar–phosphate backbone zigzags back and forth, giving rise to its name. A Z-DNA structure can result if the molecule contains particular base sequences, such as stretches of alternating C and G nucleotides. Researchers have found that Z-DNA-specific antibodies bind to regions of the DNA that are being transcribed into RNA, suggesting that Z-DNA may play some role in gene expression. Additional secondary structures of DNA (C-DNA, D-DNA, etc.) can form under specialized laboratory conditions or in DNA with specific base sequences.

THINK-PAIR-SHARE Question 5

CONCEPTS

DNA can assume different secondary structures, depending on the conditions in which it is placed and on its base sequence. B-DNA is thought to be the most common configuration in the cell.

✔ CONCEPT CHECK 8

How does Z-DNA differ from B-DNA?

CONNECTING CONCEPTS CONNECT

Genetic Implications of DNA Structure

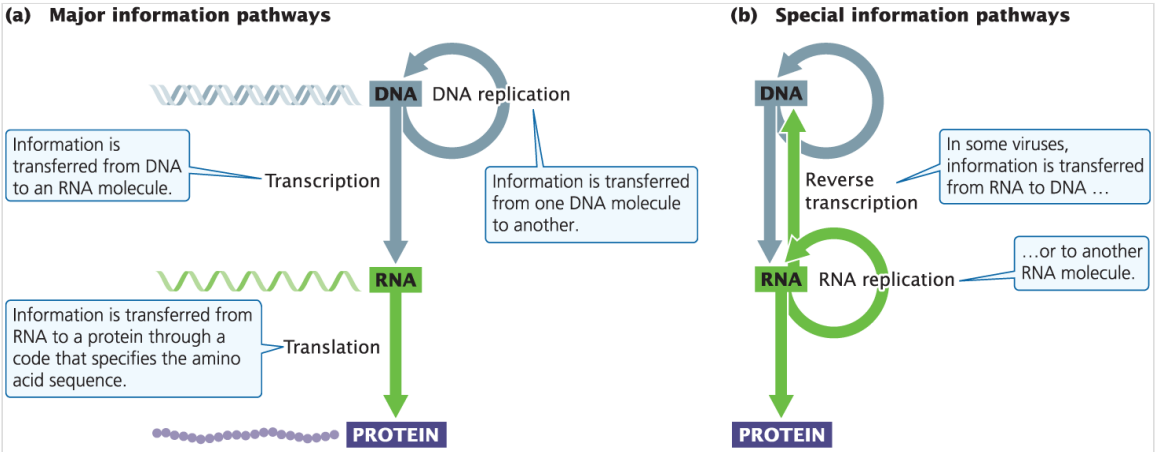
Watson and Crick's great contribution was their elucidation of the genotype's chemical structure, which made it possible for geneticists to begin to examine genes directly, instead of looking only at the phenotypic consequences of gene action. The determination of the structure of DNA led to the birth of molecular genetics—the study of the chemical and molecular nature of genetic information.

Watson and Crick's structure did more than create the potential for molecular genetic studies, however; it was an immediate source of insight into key genetic processes. At the beginning of this chapter, four fundamental requirements for genetic material were identified. Watson and Crick's structure showed how DNA met those requirements.

1. First, genetic material must be capable of carrying large amounts of information. Watson and Crick's model suggested that genetic instructions are encoded in the base sequence of DNA, the only variable part of the molecule.
2. A second necessary property of genetic material is its ability to replicate faithfully. The complementary polynucleotide strands of DNA make this replication possible. Watson and Crick proposed that in replication, the two polynucleotide strands unzip, breaking the weak hydrogen bonds between them, and each strand serves as a template on which a new strand is synthesized. The specificity of the base pairing means that only one possible sequence of bases—the complementary sequence—can be synthesized from each template strand. Newly replicated double-stranded DNA molecules are therefore identical with the original double-stranded DNA molecule (see Chapter 12 on DNA replication).
3. A third essential property of genetic material is the ability to be expressed as a phenotype. DNA expresses its genetic instructions by first transferring its information to an RNA molecule in a process termed **transcription** (see Chapter 13). The term *transcription* is appropriate because although the information is transferred from DNA to RNA, the information remains in the language of nucleic acids. In some cases, the RNA molecule then transfers the genetic information to a protein by specifying its amino acid sequence. This process is termed **translation** (see Chapter 15) because the information must be *translated* from the language of nucleotides into the language of amino acids.
4. A fourth essential property of genetic material is the capacity to vary. The variation in DNA, as we have seen, consists of differences in the sequence of bases found among different individuals.

We can now identify three major pathways of information flow in the cell (**Figure 10.17a**): in **replication**, information passes from one DNA molecule to other DNA molecules; in transcription, information passes from DNA to RNA; and in translation, information passes

from RNA to protein. This concept of information flow was formalized by Francis Crick in a concept that he called the **central dogma** of molecular biology. The central dogma states that genetic information passes from DNA to protein in a one-way information pathway. We now realize, however, that the central dogma is an oversimplification. In addition to the three general information pathways of replication, transcription, and translation, other transfers may take place in certain organisms or under special circumstances. Retroviruses (see Chapter 9) and some transposable elements (see Chapter 18) transfer information from RNA to DNA (in **reverse transcription**), and some RNA viruses transfer information from RNA to RNA (in **RNA replication**; **Figure 10.17b**).



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

10.17 Pathways of information transfer within the cell.

Description

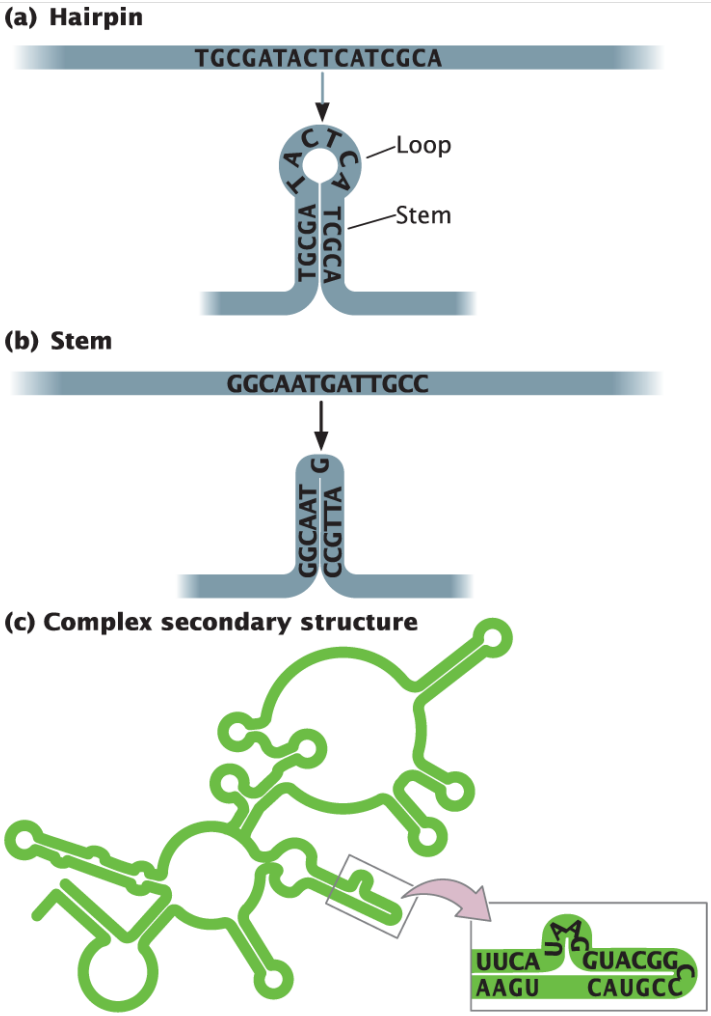
Illustration a on the left shows major information pathways. It shows a D N A helix undergoing replication to form D N A again. A caption reads, Information is transferred from one D N A molecule to another. D N A also undergoes transcription to produce an R N A strand. A caption reads, Information is transferred from D N A to an R N A molecule. Then, this R N A strand undergoes translation to form a protein. A caption reads, Information is transferred from R N A to a protein through a code that specifies the amino acid sequence.

Illustration b on the right shows special information pathways. It shows a D N A helix undergoing replication to form D N A again. D N A also undergoes transcription to produce an R N A strand. Then, this R N A strand undergoes reverse transcription to form D N A. A caption reads, In some viruses, information is transferred from R N A

to D N A or to another R N A molecule. This R N A strand further undergoes R N A replication to form R N A again. R N A strand also undergoes translation to form a protein.

10.4 Special Structures Can Form in DNA and RNA

Sequences *within* a single strand of nucleotides may be complementary to each other and able to pair by forming hydrogen bonds, producing double-stranded regions (**Figure 10.18**). This internal base pairing imparts a secondary structure to a single-stranded molecule. One common type of secondary structure found in single strands of nucleotides is a **hairpin**, which forms when sequences of nucleotides on the same strand are inverted complements (see **Figure 10.18a**). A hairpin consists of a region of paired bases (the stem) and intervening unpaired bases (a loop). When the complementary sequences are contiguous, a stem is formed with no loop (see **Figure 10.18b**). RNA molecules may contain numerous hairpins, which allow them to fold up into complex structures (see **Figure 10.18c**). Secondary structures play important roles in the functions of many RNA molecules, as we will see in Chapters 14 and 15.



10.18 Both DNA and RNA can form special secondary structures. (a) A hairpin consisting of a region of paired bases (which form the stem) and a region of unpaired bases between the complementary sequences (which form a loop at the end of the stem). (b) A stem with no loop. (c) Secondary structure of the RNA component of RNase P of *E. coli*. RNA molecules often have complex secondary structures.

Description

Illustration a at the top shows a D N A strand forming a hairpin loop structure. In the stem, the base pairs starting from 5 prime are T, G, C, G, A, and they pair with T, C, G, C, A, that start 6 base pairs away. The 6 base pairs intervening form the loop and are as follows: T, A, C, T, C, and A.

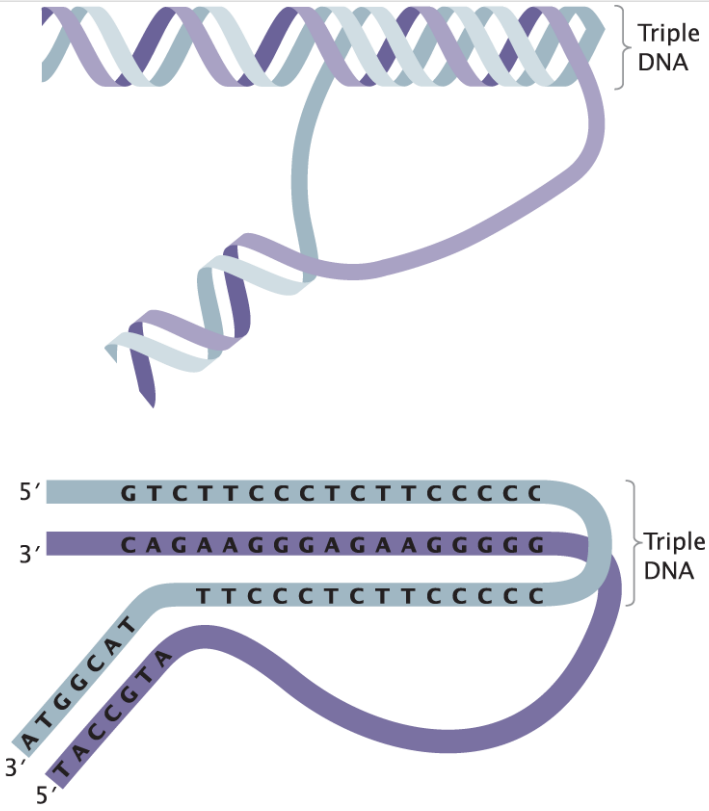
Illustration b below shows a D N A strand forming a stem structure. In the stem, 6 base pairs, starting from 5 prime G, G, C, A, A, T base pair with A, T, T, G, C, C. Only 1 base pair, G, intervenes these 2 complementary strands and sits on the top of the stem. This forms the stem.

Illustration c below shows a strand of R N A forming a complex secondary structure. The strand has 3 stems that have large loops, 9 stems with small loops, and 1 stem. Some of the stem loops have regions where a section does not base pair complement the other side of the stem and this section forms a small bump within the stem. One of these sections is magnified to show the base pairs U, U, C, A base pairing with A, A, G, U on the other side of the stem. 4 base pairs without a complement on the other side form a small bump in the stem and are as follows: U, A, A, G. The stem continues with base pairs G, U, A, C, G, G pairing with C, A, U, G, C, C with C at the top of stem.

DNA sequences can also sometimes form three-stranded (triplex) structures, called **H-DNA**, when some of the DNA unwinds and a single polynucleotide strand from one part of the molecule pairs with double-stranded DNA from another part of the molecule (**Figure 10.19**). This is possible because under certain conditions, one base can simultaneously pair with two other bases. H-DNA often occurs in long sequences containing only purine bases or only pyrimidine bases. Some triplex structures consist of one strand of purines paired with two strands of pyrimidines; other triplex structures consist of one strand of pyrimidines paired with two strands of purines. Sequences capable of adopting an H-DNA conformation are common in mammalian genomes, and evidence suggests that H-DNA occurs under natural conditions. Research has demonstrated that H-DNA breaks more readily than double-stranded DNA, which then leads to higher rates of mutation in regions where H-DNA structures occur. Quadruplex structures involving four

strands of DNA can also occur under certain conditions. ❯ **TRY PROBLEM 40**

THINK-PAIR-SHARE Question 6



10.19 H-DNA arises when three polynucleotide strands pair.

Description

The illustration shows two D N A duplexes that have a purple and grey strand. The purple strand in the first D N A duplex is continuous with the purple strand in the second duplex. The grey strand in the first duplex remains a part of the D N A strand. The grey strand from the second D N A duplex invades the first duplex for about 3 turns forming the triple D N A

strand section and replaces the purple strand in the duplex as it dissociates from that duplex.

The illustration below shows 2 grey strands sandwiching a purple strand and representing the triple D N A. The grey strands have the same D N A nucleotide sequence going left to right in the diagram. Both complement the sequence on the purple strand.

CONCEPTS

In DNA and RNA, base pairing between nucleotides on the same strand produces special secondary structures, such as hairpins. Triple-stranded DNA structures can arise when a single strand of DNA pairs with double-stranded DNA.

✔ CONCEPT CHECK 9

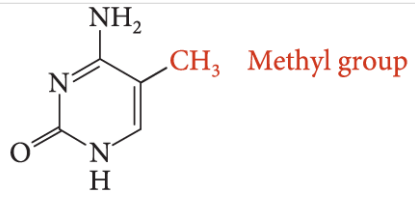
Hairpins are formed in DNA as a result of

a. sequences on the same strand that are inverted and complementary.
b. sequences on the opposite strand that are complements.
c. sequences on the same strand that are identical.
d. sequences on the opposite strand that are identical.

The primary structure of DNA can also be modified in various ways. One such modification is **DNA methylation**, a process in which methyl groups (CH_3) are added by specific enzymes to certain positions on the nitrogenous bases. Bacterial DNA is frequently methylated to distinguish it from foreign, unmethylated DNA that may be introduced by viruses; bacteria use proteins called restriction enzymes to cut up any unmethylated viral DNA (see Chapter 19). In eukaryotic cells, methylation is often related to gene expression. Sequences that are methylated typically show low levels

of transcription, while sequences lacking methylation are actively being transcribed (see Chapter 17). Methylation can also affect the three-dimensional structure of the DNA molecule and is responsible for some epigenetic effects (see Chapter 21).

Adenine and cytosine are commonly methylated in bacteria. In eukaryotic DNA, cytosine bases are sometimes methylated to form **5-methylcytosine** (**Figure 10.20**). The extent of cytosine methylation varies among eukaryotic organisms: in most animal cells, about 5% of the cytosine bases are methylated, but there is no methylation of cytosine in yeast, and more than 50% of the cytosine bases in some plants are methylated. Why eukaryotic organisms differ so widely in their degree of methylation is not clear.



5-Methylcytosine

Pierce, *Genetics: A Conceptual Approach*, 7e
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10.20 In eukaryotic DNA, cytosine bases are often methylated to form 5-methylcytosine.

CONCEPTS

Methyl groups may be added to certain bases in DNA. Both prokaryotic and eukaryotic DNA can be methylated. In eukaryotes, cytosine bases are most often methylated to form 5-methylcytosine, and methylation is often related to gene expression.

Chapter 10 Review

CHAPTER SUMMARY

Section 10.1 Learning Objective: Be able to list key characteristics of genetic material.

- Genetic material must contain complex information, be replicated accurately, code for the phenotype, and have the capacity to vary.

Section 10.2 Learning Objective: Be able to describe how researchers identified DNA as the source of genetic information.

- Evidence that DNA is the source of genetic information came from the finding by Avery, MacLeod, and McCarty that transformation depends on DNA and from the demonstration by Hershey and Chase that viral DNA is passed on to progeny phages.
- James Watson and Francis Crick, using data provided by Rosalind Franklin and Maurice Wilkins, proposed a model for the three-dimensional structure of DNA in 1953.
- The results of experiments with tobacco mosaic virus showed that RNA carries genetic information in some viruses.

Section 10.3 Learning Objective: Be able to outline the primary and secondary structures of DNA.

- A DNA nucleotide consists of a deoxyribose sugar, a phosphate group, and a nitrogenous base. An RNA nucleotide consists of a ribose sugar, a phosphate group, and a nitrogenous base.
- The bases of a DNA nucleotide are of two types: purines (adenine and guanine) and pyrimidines (cytosine and thymine). RNA contains the pyrimidine uracil instead of thymine.
- Nucleotides are joined together by phosphodiester linkages to form a polynucleotide strand. Each polynucleotide strand has a free phosphate group at its $5'$ end and a free hydroxyl group at its $3'$ end.
- DNA consists of two nucleotide strands that wind around each other to form a double helix. The sugars and phosphates lie on the outside of the helix, and the bases are stacked in the interior. The two strands are joined together by hydrogen bonding between bases in each strand. The two strands are antiparallel and complementary.
- DNA molecules can form a number of different secondary structures, depending on the conditions in which the DNA is placed and on its base sequence.
- The structure of DNA has several important genetic implications. Genetic information resides in the base sequence of DNA, which ultimately specifies the amino acid sequence of proteins. Complementarity of the bases on DNA's two strands allows genetic information to be replicated.

- The central dogma of molecular biology proposes that information flows in a one-way direction, from DNA to RNA to protein. Exceptions to the central dogma are now known.

Section 10.4 Learning Objective: Be able to describe special structures that occur within DNA and RNA.

- Pairing between bases on the same nucleotide strand can lead to hairpins and other special secondary structures.
- DNA may be modified by the addition of methyl groups to the nitrogenous bases.

WORKED PROBLEMS

Problem 1

The percentage of cytosine in a double-stranded DNA molecule is 40%. What is the percentage of thymine?

» Solution Strategy

What information is required in your answer to the problem?

The percentage of thymine in the DNA molecule.

What information is provided to solve the problem?

- The DNA molecule is double stranded.
- The percentage of cytosine is 40%.

> **Recall:** In double-stranded DNA, A pairs with T, whereas G pairs with C; so the percentage of A equals the percentage of T, and the percentage of G equals the percentage of C.

For help with this problem, review:

The Primary Structure of DNA and Secondary Structures of DNA in Section 10.3.

» Solution Steps

If C=40%, then G also must be 40%. The total percentage of C + G is therefore 40% + 40% = 80%. All the remaining bases must be either A or T, so the total percentage of A + T = 100% − 80% = 20%; because the percentage of A equals the percentage of T, the percentage of T is 20% / 2 = 10%.

Problem 2

Which of the following relations will be true for the percentage of bases in double-stranded DNA?

a. C + T = A + G
b. $\frac{C}{A} = \frac{T}{G}$

» Solution Strategy

What information is required in your answer to the problem?

Whether C + T = A + G and $\frac{C}{A} = \frac{T}{G}$ are true.

What information is provided to solve the problem?

- The DNA is double stranded.
- Ratios of different groups of bases.

For help with this problem, review:

The Primary Structure of DNA and Secondary Structures of DNA in Section 10.3.

» Solution Steps

An easy way to determine whether the relations are true is to arbitrarily assign percentages to the bases, remembering that, in double-stranded DNA, A = T and G = C. For example, if the percentages of A and T are each 30%, then the percentages of G and C are each 20%. We can substitute these values into the equations to see if the relations are true.

a. 20 + 30 = 30 + 20. This relation is true.
b. $^{20}/_{30} \neq {}^{30}/_{20}$. This relation is not true.

IMPORTANT TERMS

nucleotide
Chargaff's rules
transforming principle
isotope
X-ray diffraction
ribose
deoxyribose
nitrogenous base
purine
pyrimidine
adenine (A)
guanine (G)
cytosine (C)
thymine (T)
uracil (U)
nucleoside
phosphate group
deoxyribonucleotide
ribonucleotide
phosphodiester linkage
polynucleotide strand
5′ end
3′ end
antiparallel
complementary DNA strands

B-DNA
A-DNA
Z-DNA
transcription
translation
replication
central dogma
reverse transcription
RNA replication
hairpin
H-DNA
DNA methylation
5-methylcytosine

ANSWERS TO CONCEPT CHECKS

1. Without knowledge of the structure of DNA, an understanding of how genetic information was encoded or expressed was impossible.
2. c
3. c
4. No, because carbon is found in both protein and nucleic acid.
5. d
6. b
7. d
8. Z-DNA has a left-handed helix; B-DNA has a right-handed helix. The sugar–phosphate backbone of Z-DNA zigzags back and

forth, whereas the sugar–phosphate backbone of B-DNA forms a smooth, continuous ribbon.

9. a

Chapter 10 Assessment

COMPREHENSION QUESTIONS

Section 10.1

1. What four general characteristics must the genetic material possess?

Section 10.2

2. Briefly outline the history of our knowledge of the structure of DNA until the time of Watson and Crick. Which do you think were the principal contributions and developments?

3. What experiments demonstrated that DNA is the genetic material?

4. What is transformation? How did Avery and his colleagues demonstrate that the transforming principle is DNA?

5. How did Hershey and Chase show that DNA is passed to new phages in phage reproduction?

6. Why was the discovery of DNA structure so important?

Section 10.3

7. Draw and identify the three parts of a DNA nucleotide.

8. How does an RNA nucleotide differ from a DNA nucleotide?
9. How does a purine differ from a pyrimidine? What purines and pyrimidines are found in DNA and RNA?
10. Draw a short segment of a single polynucleotide strand, including at least three nucleotides. Indicate the polarity of the strand by identifying the $5'$ end and the $3'$ end.
11. Which bases are capable of forming hydrogen bonds with each other?
12. What different types of chemical bonds are found in DNA, and where are they found?
13. What are some of the important genetic implications of the DNA structure?
14. What are the three major pathways of information flow within the cell?

Section 10.4

15. What are hairpins and how do they form?
16. What is DNA methylation?

APPLICATION QUESTIONS AND PROBLEMS

Introduction

17. The introduction to this chapter, which describes the sequencing of 90,000-year-old DNA, emphasizes DNA's extreme stability. What aspects of DNA's structure contribute to the stability of the molecule? Why is RNA less stable than DNA?

Section 10.2

18. *Match the researchers (a–j) with the discoveries listed.

    a. Kossel

    b. Fraenkel-Conrat

    c. Watson and Crick

    d. Levene

    e. Miescher

    f. Hershey and Chase

    g. Avery, MacLeod, and McCarty

    h. Griffith

    i. Franklin and Wilkins

    j. Chargaff

    _____ Took X-ray diffraction pictures used in constructing the structure of DNA.
    _____ Determined that DNA contains nitrogenous bases.
    _____ Identified DNA as the genetic material in bacteriophages.

_____ Discovered regularity in the ratios of different bases in DNA.
_____ Determined that DNA is responsible for transformation in bacteria.
_____ Worked out the helical structure of DNA by building models.
_____ Discovered that DNA consists of repeating nucleotides.
_____ Determined that DNA is acidic and high in phosphorus.
_____ Conducted experiments showing that RNA can serve as the genetic material in some viruses.
_____ Demonstrated that heat-killed material from bacteria can genetically transform live bacteria.

19. *A student mixes some heat-killed type IIS *S. pneumoniae* bacteria with live type IIR bacteria and injects the mixture into a mouse. The mouse develops pneumonia and dies. The student recovers some type IIS bacteria from the dead mouse. If this is the only experiment conducted by the student, has the student demonstrated that transformation has taken place? What other explanations might explain the presence of the type IIS bacteria in the dead mouse?

20. Predict what would happen if Griffith had mixed some heat-killed type IIIS bacteria and some heat-killed type IIR bacteria and injected these into a mouse. Would the mouse have contracted pneumonia and died? Explain why or why not.

21. CONNECT Explain how heat-killed type IIIS bacteria in Griffith's experiment genetically altered the live type IIR bacteria. (Hint: See the discussion of transformation in Chapter 9.)

22. What results would you expect if the bacteriophage that Hershey and Chase used in their experiment had contained RNA instead of DNA?

23. Which of the processes of information transfer illustrated in **Figure 10.17** are required for the T2 phage reproduction illustrated in **Figure 10.5**?

24. *Imagine that you are a student in Alfred Hershey and Martha Chase's lab in the late 1940s. You are given five test tubes containing *E. coli* bacteria infected with T2 bacteriophages that have been labeled with either ^{32}P or ^{35}S. Unfortunately, you forget to mark the tubes and are now uncertain about which were labeled with ^{32}P and which with ^{35}S. You place the contents of each tube in a blender and turn it on for a few seconds to shear off the phage protein coats. You then centrifuge the contents to separate the protein coats from the cells. You check for the presence of radioactivity and obtain the following results. Which tubes contained *E. coli* infected with ^{32}P-labeled phage? Explain your answer.

Tube number	Radioactivity present in
1	Cells
2	Protein coats
3	Protein coats
4	Cells
5	Cells

25. **Figure 10.9** illustrates Fraenkel-Conrat and Singer's experiment on the genetic material of TMV. What results would you expect in this

experiment if protein carried the genetic information of TMV instead of RNA?

Section 10.3

26. *DNA molecules of different sizes are often separated with the use of a technique called electrophoresis (see Chapter 19). With this technique, DNA molecules are placed in a gel, an electrical current is applied to the gel, and the DNA molecules migrate toward the positive (+) pole of the current. What aspect of its structure causes a DNA molecule to migrate toward the positive pole?

27. *Each nucleotide pair of a DNA double helix weighs about 1×10^{-21}g. The human body contains approximately 0.5 g of DNA. How many nucleotide pairs of DNA are in the human body? If you assume that all the DNA in human cells is in the B-DNA form, how far would the DNA reach if stretched end to end?

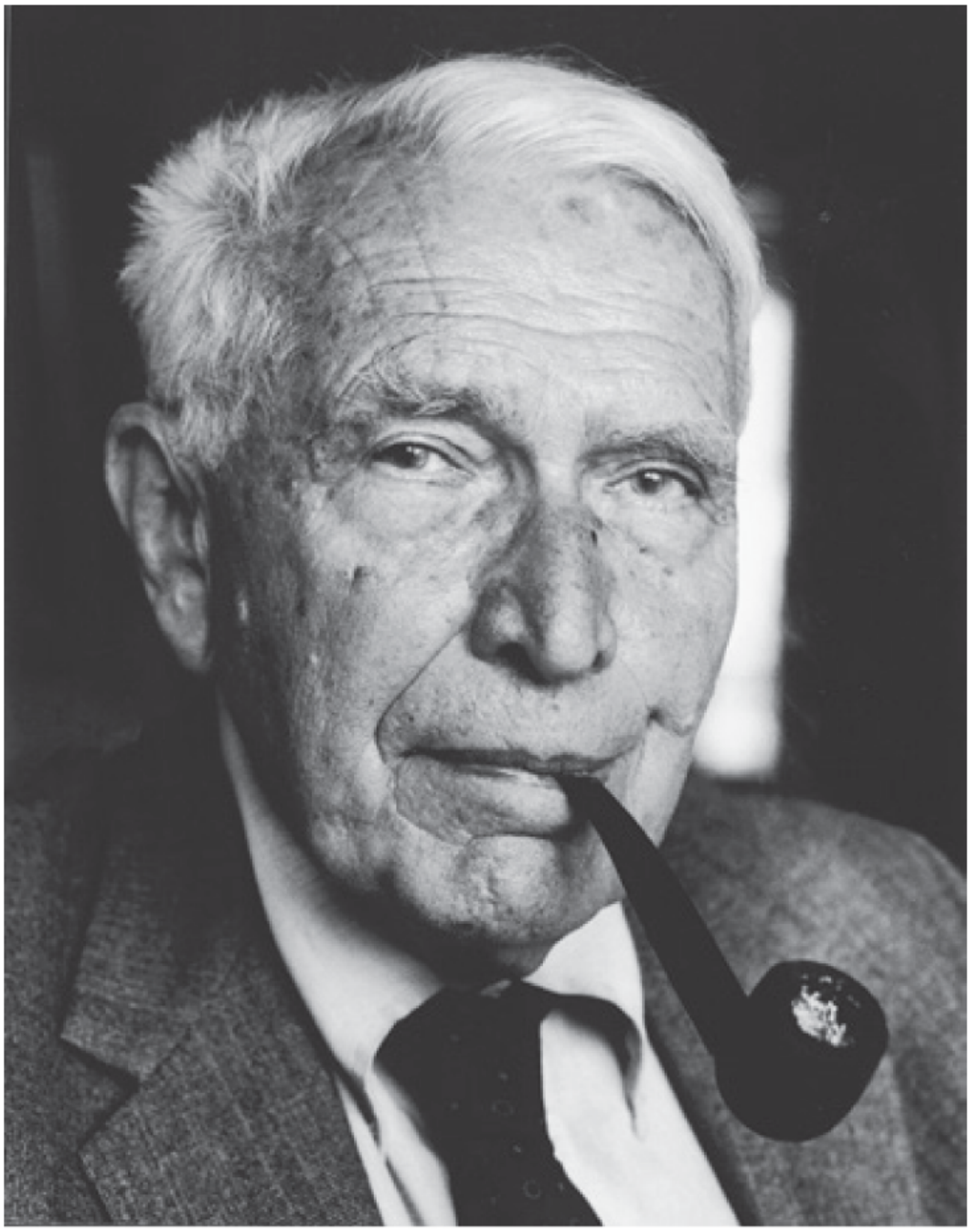
28. One nucleotide strand of a DNA molecule has the base sequence illustrated below.

    $5'$–ATTGCTACGG–$3'$

    Give the base sequence, and label the $5'$ and $3'$ ends of the complementary DNA nucleotide strand.

29.

*Erwin Chargaff collected data on the proportions of nitrogenous bases from the DNA of a variety of different organisms and tissues (E. Chargaff, in *The Nucleic Acids: Chemistry and Biology*, vol. 1, E. Chargaff and J. N. Davidson, Eds. New York: Academic Press, 1955). The following data are from the DNA of several organisms analyzed by Chargaff.



Horst Tappe/Getty Images.

Erwin Chargaff.

Percentage

Organism and tissue	A	G	C	T
Sheep thymus	29.3	21.4	21.0	28.3
Pig liver	29.4	20.5	20.5	29.7
Human thymus	30.9	19.9	19.8	29.4
Rat bone marrow	28.6	21.4	20.4	28.4
Hen erythrocytes	28.8	20.5	21.5	29.2
Yeast	31.7	18.3	17.4	32.6
E. coli	26.0	24.9	25.2	23.9
Human sperm	30.9	19.1	18.4	31.6
Salmon sperm	29.7	20.8	20.4	29.1
Herring sperm	27.8	22.1	20.7	27.5

a. For each organism, compute the ratio of (A + G)/(T + C) and the ratio of (A + T)/(C + G).

b. Are these ratios constant, or do they vary among the organisms? Explain why.

c. Is the (A + G)/(T + C) ratio different for the sperm samples? Would you expect it to be? Why or why not?

30. Boris Magasanik collected data on the amounts of the bases of RNA isolated from a number of sources, expressed relative to a value of 10 for adenine (B. Magasanik, in *The Nucleic Acids: Chemistry and Biology*, vol. 1, E. Chargaff and J. N. Davidson, Eds. New York: Academic Press, 1955).

	Amount			
Organism and tissue	**A**	**G**	**C**	**U**
Rat liver nuclei	10	14.8	14.3	12.9
Rabbit liver nuclei	10	13.6	13.1	14.0
Cat brain	10	14.7	12.0	9.5
Carp muscle	10	21.0	19.0	11.0
Yeast	10	12.0	8.0	9.8

a. For each organism, compute the ratio of (A + G)/(U + C).

b. How do these ratios compare with the (A + G)/(T + C) ratio found in DNA (see Problem 29)? Explain.

31. Which of the following relations or ratios would be true for a double-stranded DNA molecule?

a. A + T = G + C

b. A + G = T + C

c. A + C = G + T

d. $\frac{A + T}{C + G} = 1.0$

e. $\frac{A + G}{C + T} = 1.0$

f. $\frac{A}{C} = \frac{G}{T}$

g. $\frac{A}{G} = \frac{C}{T}$

h. $\frac{A}{T} = \frac{G}{C}$

32. *If a double-stranded DNA molecule is 15% thymine, what are the percentages of all the other bases?

33.

In pea plants, tall (*T*) is dominant to dwarf (*t*). Let us assume that the DNA sequence for the tall allele is TTTT and the sequence that codes for the dwarf allele is TGTT. A geneticist crosses a pea plant homozygous for tall with a dwarf plant to produce F_1 progeny (see Chapter 3 for a review of genetic crosses). Draw a picture that illustrates the DNA molecules that code for tall and dwarf alleles in a pair of chromosomes of the F_1 progeny from this cross at the following stages. Label the DNA sequences for the tall and dwarf alleles. To keep the diagram simple, you can use a straight line for each polynucleotide chain.

a. G_1 of interphase

b. Metaphase I of meiosis

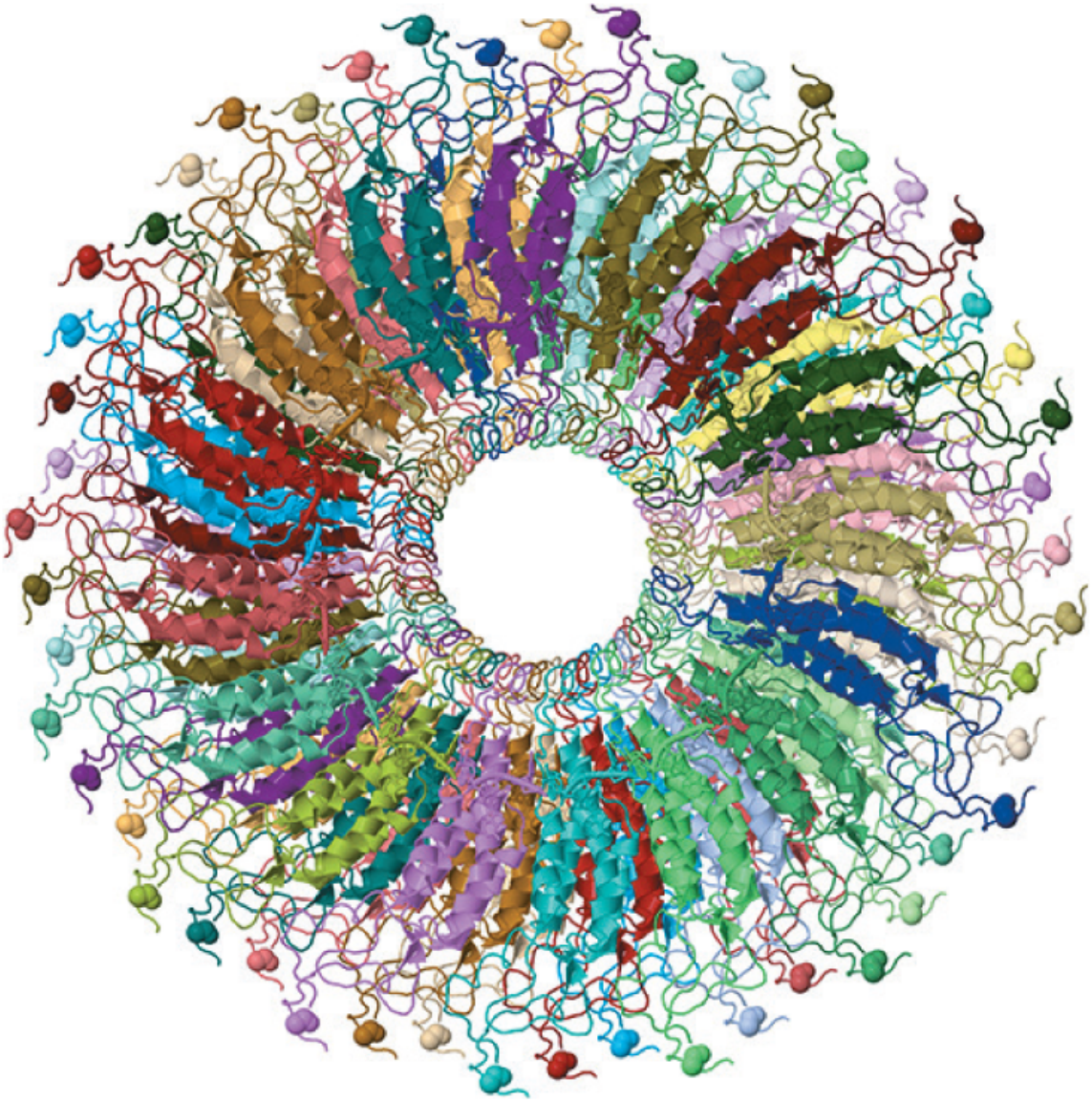
c. Anaphase II of meiosis

34. Suppose that each of the bases in DNA were capable of pairing with any other base. What effect would this capability have on DNA's capacity to serve as the source of genetic information?

35.

Heinz Shuster collected the following data on the base composition of ribgrass mosaic virus (H. Shuster, in *The Nucleic Acids: Chemistry and Biology*, vol. 3, E. Chargaff and J. N. Davidson, Eds. New York: Academic Press, 1955). On the basis of this information, is the hereditary information of the ribgrass mosaic virus RNA or DNA? Is it likely to be single stranded or double stranded?

	Percentage				
	A	**G**	**C**	**T**	**U**
Ribgrass mosaic virus	29.3	25.8	18.0	0.0	27.0



Leibniz Institute for Age Research, Fritz Lipmann-Institute.

Ribgrass mosaic virus.

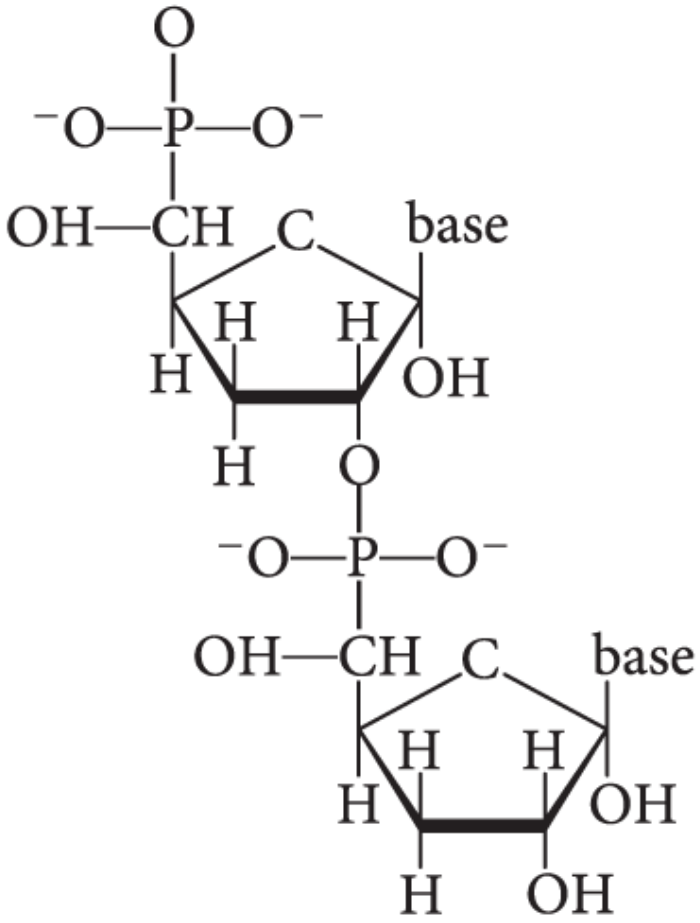
36. *The relative amounts of each nitrogenous base are tabulated here for four different viruses. For each virus listed in the following table, indicate whether its genetic material is DNA or RNA and

whether it is single stranded or double stranded. Explain your reasoning.

Virus	T	C	U	G	A
I	0	12	9	12	9
II	23	16	0	16	23
III	34	42	0	18	39
IV	0	24	35	27	17

37. *A B-DNA molecule has 1 million nucleotide pairs. How many complete turns of the helix are there in this molecule?

38. *For entertainment on a Friday night, a genetics professor proposed that his children diagram a polynucleotide strand of DNA. Having learned about DNA in preschool, his 5-year-old daughter was able to draw a polynucleotide strand, but she made quite a few mistakes. The daughter's diagram (represented here) contained at least 10 mistakes.



Description
The structure begins with a phosphate group that has a central P atom, having 2 O' negatives and one O atom. The P with 3 oxygen atoms is bound to a sugar molecule through the 5 prime C atom. The three other groups attached to 5 prime C are a hydroxyl group (O H), a hydrogen (H), and the 4 prime C of the pentagon. 4 prime and 3 prime C's are individually bonded to a hydrogen atom. C prime 1 is attached to a base and a hydroxyl group (O H). The 2 prime C is attached to the O atom of the adjoining phosphate group attached. This phosphate group is same as the above phosphate group C prime 1 is attached to a base and a hydroxyl group (O H).

a. Make a list of all the mistakes in the structure of this DNA polynucleotide strand.

b. Draw the correct structure for the polynucleotide strand.

39. CONNECT Chapter 1 considered the theory of the inheritance of acquired characteristics and noted that this theory is no longer accepted. Is the central dogma consistent with the theory of the inheritance of acquired characteristics? Why or why not?

Section 10.4

40. *Write a sequence of bases in an RNA molecule that will produce a hairpin structure.

CHALLENGE QUESTIONS

Section 10.1

41. *Suppose that an automated, unmanned probe is sent into deep space to search for extraterrestrial life. After wandering for many light-years among the far reaches of the universe, this probe arrives on a distant planet and detects life. The chemical composition of life on this planet is completely different from that of life on Earth, and its genetic material is not composed of nucleic acids. What predictions can you make about the chemical properties of the genetic material on this planet?

Section 10.2

42. How might ^{32}P and ^{35}S be used to demonstrate that the transforming principle is DNA? Briefly outline an experiment that

would show that DNA, rather than protein, is the transforming principle.

Section 10.3

43. Researchers have proposed that early life on Earth used RNA as its source of genetic information and that DNA eventually replaced RNA as the source of genetic information. What aspects of DNA structure might make it better suited than RNA to be the genetic material?

44. Scientists reportedly isolated short fragments of DNA from fossilized dinosaur bones hundreds of millions of years old. The technique used to isolate this DNA is the polymerase chain reaction, which is capable of amplifying very small amounts of DNA a millionfold (see Chapter 19). Critics claimed that the DNA isolated from dinosaur bones is not purely of ancient origin but instead has been contaminated by DNA from present-day organisms, such as bacteria, mold, or humans. What precautions, analyses, and control experiments could be carried out to ensure that DNA recovered from fossils is truly of ancient origin?

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 10.1

1. Suppose that proteins, instead of nucleic acids, had evolved as the carriers of genetic information. How well would proteins satisfy the four requirements for the genetic material listed in Section 10.1?

Section 10.2

2. Isaac Newton said, "If I have seen further, it is by standing on the shoulders of giants." How does this statement apply to Watson and Crick?

3. Compare and contrast Gregor Mendel's scientific method and approach to science (see Chapter 3) with that of Watson and Crick. How did they differ? Were there any similarities?

Section 10.3

4. Draw a short section of DNA. Include both nucleotide strands and at least two nucleotides for each strand. Your drawing should include the structure of the nucleotides, as well as show how nucleotides are joined together to form a polynucleotide strand and how the two nucleotide strands are connected.

5. How does its structure enable DNA to function effectively as the genetic material? Give some specific examples.

6. Write out the sequence of a theoretical DNA molecule 50 bp in length that consists of 12% guanine and, when one strand is copied into RNA, produces an RNA molecule that has a 14 bp hairpin structure.

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. What was the genetic origin of Denny, a woman from the Denisova cave who lived 90,000 years ago? Create a concept map to explain Denny's origin and how scientists verified intimate details of Denny's family life using the extreme stability of DNA.

Section 10.2

2. Draw a concept map that summarizes the key discoveries that led to the conclusion that DNA was the genetic material.

Section 10.3

3. What is the structure of DNA? Create a concept map that outlines the basic structure of DNA using the following terms: DNA, polynucleotide strand, purine, pyrimidine, antiparallel, base, phosphate group, adenine, thymine, guanine, cytosine, nucleotides, nucleosides, complementary, deoxyribose sugar.

Section 10.4

4. Draw a concept map that summarizes the major information pathways and special information pathways in cells. In your map, compare and contrast the major information pathways with special information pathways.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 11

Chromosome Structure and Organelle DNA

Romano Cagnoni/Getty Images.

Children from a Romanian orphanage. Research demonstrated that children who lived in state-run orphanages had shorter telomeres than children raised in foster homes.

Telomeres and Childhood Adversity

Within each of our cells are 46 chromosomes, exquisitely complex structures of DNA and protein that carry the coding instructions for all of our traits. These chromosomes are passed down from our parents and constitute the basis of heredity, the passage of traits from one generation to the next. But chromosomes don't just carry a record of our genetic legacy. They also carry a record—in the lengths of their telomeres—of the stresses we encounter.

Telomeres are special protective structures found at the ends of each of our chromosomes. Like the small plastic tips that keep the ends of a shoelace from unraveling, telomeres prevent chromosomes from being degraded at their ends. In spite of the protection of the telomeres, chromosomes of most cells shorten progressively with each cell division. Due to a quirk of DNA replication, most cells are unable to copy the very end of each linear chromosome (see Chapter 12 for a full discussion of the end-replication problem). Hence, with each round of replication, a chromosome becomes shorter, until it is so reduced that the cell stops dividing, becomes inactive, and eventually dies. For most cells, this shortening of telomeres limits the number of divisions possible. Exceptions occur in the germ-line cells that produce future generations, certain stem cells, and—unfortunately—many cancer cells that have escaped normal constraints on cell division.

Because telomeres become shorter with each cell division, much research has focused on determining if telomere length is indicative of biological aging. Although the relation between telomere length

and aging is complex and not fully understood, considerable evidence suggests that telomeres do shorten with age, and that processes that lead to premature telomere shortening are associated with features of aging. In 2011, geneticists studying this phenomenon observed that hardships encountered early in life can play a part in shortening our telomeres.

To study the effects of early life experience on telomere length, geneticists studied 100 children living in state-run orphanages in Romania. At an early age, some of these children were placed in foster homes; others remained in the orphanages. Previous studies had demonstrated that children in such orphanages receive less individual attention and care compared with children growing up with natural or foster parents, and institutional care is assumed to be more stressful than foster care.

When the children were 6 to 10 years old, the researchers collected samples of their DNA and measured the length of their telomeres. The results were striking: children who remained in the orphanages had significantly shorter telomeres than those who spent time in foster care. The researchers concluded that telomere length is affected by childhood adversity: children reared in stressful environments are more likely to have shorter telomeres than those raised in less stressful environments. Several other studies have found a similar association between telomere length in adults and early childhood stresses, such as abuse and chronic illness. How stress affects telomeres and results in their shortening is not

known, but the research documents that chromosomes are more than just a repository of our genetic information: their structure is also affected by our environment.

THINK-PAIR-SHARE

- Most cells are unable to copy the ends of chromosomes, and therefore chromosomes shorten with each cell division. This limits the number of times a cell can divide. In germ cells and stem cells, however, an enzyme called telomerase lengthens the telomeres and prevents chromosome shortening. Thus, these cells are not limited in the number of times they can divide. All cells have the gene for telomerase, but most somatic cells don't express it, and they produce no telomerase. Why don't somatic cells express telomerase and have unlimited division?
- The introduction to this chapter discussed recent research showing that children who experience early childhood stresses have shorter telomeres. How might this information be used in a practical sense?

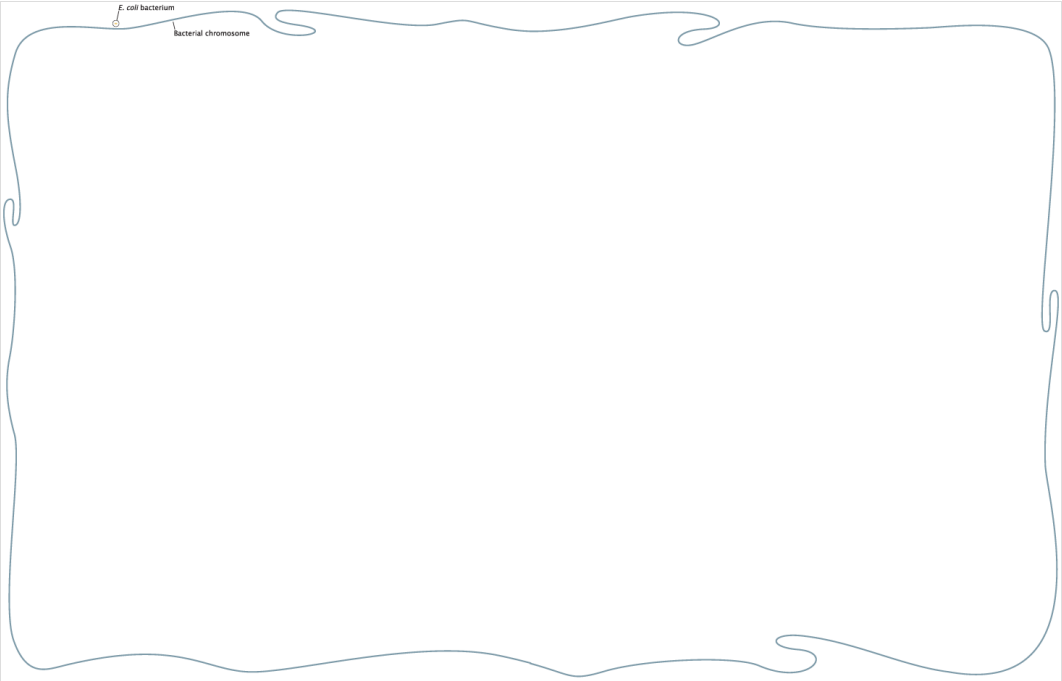
In this chapter, we examine the molecular structure of chromosomes and of the DNA found in cytoplasmic organelles. The first part of the chapter focuses on a storage problem: how to cram tremendous amounts of DNA into the limited confines of a cell. Even in those organisms that have the smallest amounts of DNA, the length of the genetic material far exceeds the length of the cell. Thus, cellular DNA must be highly folded and tightly packed. But this packing itself creates problems: it renders the DNA inaccessible, unable to be copied or read. Functional DNA must be capable of partly unfolding and expanding so that individual genes can undergo replication and transcription. The flexible, dynamic nature of DNA packing that allows it to meet these challenges is a

major theme of this chapter. We first consider supercoiling, an important tertiary structure of DNA found in both prokaryotic and eukaryotic cells. After a brief look at the bacterial chromosome, we examine the structure of eukaryotic chromosomes. We pay special attention to the working parts of a chromosome—specifically, centromeres and telomeres. We also consider the types of DNA sequences present in many eukaryotic chromosomes.

The second part of this chapter focuses on the organization of DNA sequences found in mitochondria and chloroplasts. The uniparental pattern of inheritance exhibited by genes found in these organelles was discussed in Chapter 5; here, we examine molecular aspects of organelle DNA. We briefly consider the structures of mitochondria and chloroplasts, the inheritance of traits encoded by their genes, and the evolutionary origin of these organelles. We then examine the general characteristics of **mitochondrial DNA (mtDNA)**, followed by a discussion of the organization and function of different types of mitochondrial genomes. Finally, we turn to **chloroplast DNA (cpDNA)** and examine its characteristics, organization, and function.

11.1 Large Amounts of DNA Are Packed into a Cell

The packaging of tremendous amounts of genetic information into the small space within a cell has been called the ultimate storage problem. Consider the chromosome of the bacterium *Escherichia coli*, a single molecule of DNA with approximately 4.6 million base pairs. Stretched out straight, this DNA would be about 1000 times as long as the cell it resides in (Figure 11.1). Human cells contain more than 6 billion base pairs of DNA, which would measure over 2 m (over 6 feet) stretched end to end. Even the DNA in the smallest human chromosome would stretch 14,000 times the length of the cell nucleus. Clearly, DNA molecules must be tightly packed to fit into such small spaces.



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11.1 The DNA in *E. coli* is about 1000 times as long as the cell itself.

Description
The bacterium is a small and circular shaped structure. The bacterial chromosome is a thin thread-like structure that is very long as compared to the size of E coli.

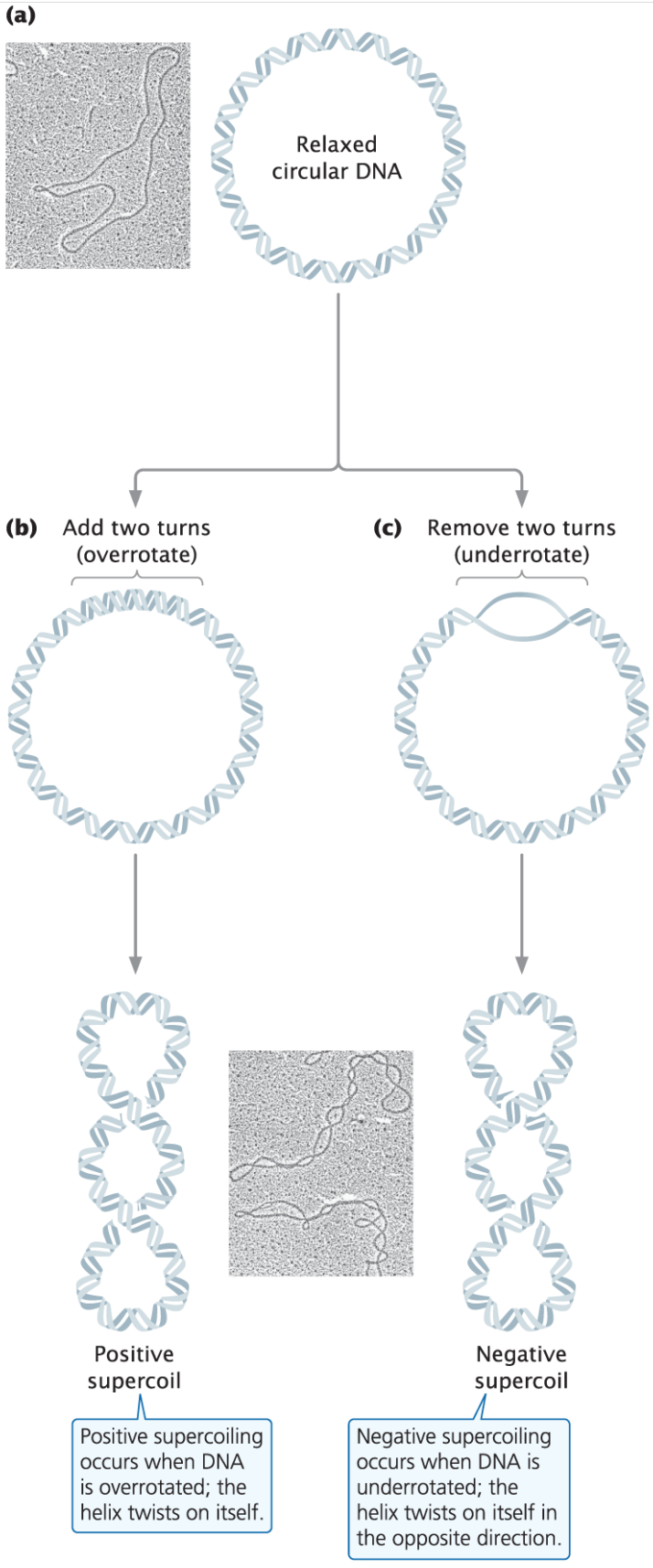
The structure of DNA can be considered at three hierarchical levels: the primary structure of DNA is its nucleotide sequence; the secondary structure is the double-stranded helix; and the tertiary structure is the higher-order folding that allows DNA to be packed into the confined space of a cell. The primary and secondary structures of DNA were described in Chapter 10; in this section, our focus is on its tertiary structure.

CONCEPTS

Chromosomal DNA exists in the form of very long molecules that are tightly packed to fit into the small confines of a cell.

Supercoiling

One type of DNA tertiary structure is **supercoiling**, which takes place when the DNA helix is subjected to strain by being overwound or underwound. B-DNA (see Chapter 10) is in its lowest-energy state when it has approximately 10 bp per turn of its helix. In this **relaxed state**, a stretch of 100 bp of DNA would assume about 10 complete turns (**Figure 11.2a**). If energy is used to add or remove any turns, strain is placed on the molecule, causing the helix to supercoil, or twist on itself. Molecules that are overrotated exhibit **positive supercoiling** (**Figure 11.2b**). Underrotated molecules exhibit **negative supercoiling** (**Figure 11.2c**). Supercoiling is a partial solution to the cell's DNA packing problem because supercoiled DNA occupies less space than relaxed DNA.



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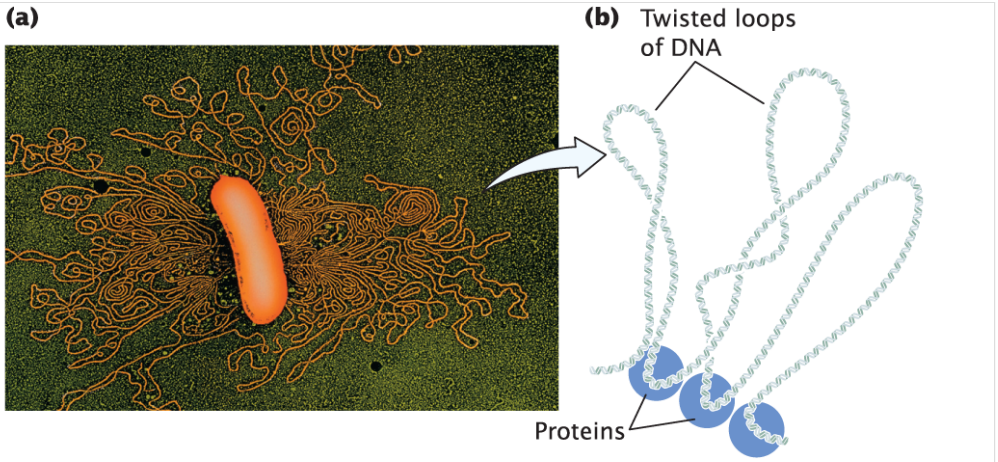
Photographs: Gopal Murti/Medical Images.

11.2 Supercoiled DNA is overwound or underwound, causing it to twist on itself.
Electron micrographs are of relaxed DNA (top) and supercoiled DNA (bottom).

Description
Illustration a at the top left shows an electron micrograph of a circular, relaxed circular D N A and a photo of a relaxed telephone cord at the top right. A caption reads, a telephone cord is like relaxed circular D N A. Illustration b at the bottom left shows the circular D N A after adding 2 turns (overrotate). This leads to the helix twisting onto itself. A caption reads, Positive supercoiling occurs when D N A is over-rotated; the helix twists on itself. An electron micrograph shows supercoiled D N A. Illustration c at the bottom right shows removing two turns or under-rotating the D N A. This leads to negative supercoiling where the helix twists onto itself but in the opposite direction. A caption reads, negative supercoiling occurs when D N A is under-rotated; the helix twists on itself in the opposite direction. A photo at the bottom right shows a telephone wire twisting on itself. A caption reads, if you turn the receiver when you hang up, you induce a negative supercoil in the cord.

Supercoiling takes place when the strain of overrotating or underrotating cannot be compensated by the turning of the ends of the double helix, which is the case if the DNA is circular—that is, there are no free ends. If the ends *can* turn freely, they will simply turn as extra rotations are added or removed, and the molecule will spontaneously revert to the relaxed state. Both bacterial and eukaryotic DNA usually fold into loops stabilized by proteins (which prevent free rotation of the ends; see Figure 11.3), and supercoiling takes place within the loops.



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Part a: G. Murti/Science Source.

11.3 Bacterial DNA is highly folded into a series of twisted loops.

Description
The photo labeled a on the left shows a rod-shaped bacterium surrounded by twisted loops of bacterial D N A and an illustration labeled b on the right shows a magnified view of the twisted loops of D N A that are attached to three round proteins molecules at the bottom.

Supercoiling relies on **topoisomerases**, enzymes that add or remove rotations from the DNA helix by temporarily breaking the nucleotide strands, rotating the ends around each other, and then rejoining the broken ends. Thus, topoisomerases can both induce and relieve supercoiling, although not all topoisomerases do both.

Most DNA found in cells is negatively supercoiled. This state has two advantages over relaxed DNA. First, negative supercoiling makes the separation of the two strands of DNA easier during replication and transcription. Negatively supercoiled DNA is underrotated, so separation of the two strands during replication and transcription is

more rapid and requires less energy. Second, the supercoiled DNA can be packed into a smaller space than can relaxed DNA.

CONCEPTS

Overrotation or underrotation of a DNA double helix places strain on the molecule, causing it to supercoil. Supercoiling is controlled by topoisomerase enzymes. Most cellular DNA is negatively supercoiled, which eases the separation of nucleotide strands during replication and transcription and allows the DNA to be packed into small spaces.

✔ CONCEPT CHECK 1

A DNA molecule 300 bp long has 20 complete rotations. This DNA molecule is

a. positively supercoiled.
b. negatively supercoiled.
c. relaxed.

The Bacterial Chromosome

Most bacterial genomes consist of a single circular DNA molecule, although linear DNA molecules have been found in a few species. In circular bacterial chromosomes, the DNA does not exist in an open, relaxed circle; the 3 million to 4 million base pairs of DNA found in a typical bacterial genome would be much too large in this state to fit into a bacterial cell (see Figure 11.1). Unlike eukaryotic DNA, bacterial DNA is not attached to histone proteins, but bacterial DNA is associated with a number of proteins that help to compact it.

When a bacterial cell is viewed with an electron microscope, its DNA frequently appears as a distinct clump, called the **nucleoid**, which is confined to a definite region of the cytoplasm. If a bacterial cell is broken open gently, its DNA spills out in a series of twisted loops (**Figure 11.3a**). The ends of the loops are most likely held in place by proteins (**Figure 11.3b**). Many bacteria contain additional DNA in the form of small circular molecules called plasmids, which replicate independently of the chromosome (see Chapter 9).

CONCEPTS

A typical bacterial chromosome consists of a large circular molecule of DNA that forms a series of twisted loops. Within the cell, bacterial DNA appears as a distinct clump, called the nucleoid.

✔ CONCEPT CHECK 2

How does bacterial DNA differ from eukaryotic DNA?

Eukaryotic Chromosomes

Individual eukaryotic chromosomes contain enormous amounts of DNA. Each eukaryotic chromosome consists of a single extremely long linear molecule of DNA. For this DNA to fit into the nucleus, tremendous packing and folding are required, the extent of which must change in the course of the cell cycle. The chromosomes are in

an elongated, relatively uncondensed state during interphase (see Figure 2.10), but the term *relatively* is important here. Although the DNA of interphase chromosomes is less tightly packed than the DNA of mitotic chromosomes, it is still highly condensed; it's just *less* condensed. In the course of the cell cycle, the level of DNA packing changes: chromosomes progress from a highly packed state to a state of extreme condensation, which is necessary for chromosome movement in mitosis and meiosis. DNA packing also changes locally during replication and transcription, when the two nucleotide strands must unwind so that particular base sequences are exposed. Thus, the packing of eukaryotic DNA (its tertiary chromosomal structure) is not static but changes regularly in response to cellular processes.

CHROMATIN

Eukaryotic DNA in the cell is closely associated with proteins. This complex of DNA and proteins is called *chromatin*. The two basic types of chromatin are **euchromatin**, which undergoes the normal process of condensation and decondensation in the cell cycle, and **heterochromatin**, which remains in a highly condensed state throughout the cell cycle, even during interphase. Euchromatin constitutes the majority of the chromosomal material and is where most transcription takes place. All chromosomes have permanent heterochromatin (called *constitutive heterochromatin*) at the centromeres and telomeres; the Y chromosome also consists largely

of constitutive heterochromatin. Heterochromatin may also occur during certain developmental stages; this material is referred to as *facultative heterochromatin*. For example, facultative heterochromatin occurs along one entire X chromosome in female mammals when that X becomes inactivated (see Chapter 4). In addition to remaining condensed throughout the cell cycle, heterochromatin is characterized by a general lack of transcription, the absence of crossing over, and replication late in the S phase. Differences between euchromatin and heterochromatin are summarized in **Table 11.1**.

THINK-PAIR-SHARE Question 1

TABLE 11.1 Characteristics of euchromatin and heterochromatin

Characteristic	Euchromatin	Heterochromatin
Chromatin condensation	Less condensed	More condensed
Location	On chromosome arms	At centromeres, telomeres, and other specific places
Type of sequences	Unique sequences	Repeated sequences*

Presence of genes	Many genes	Few genes*
When replicated	Throughout S phase	Late S phase
Transcription	Often	Infrequent
Crossing over	Common	Uncommon

*Applies only to constitutive heterochromatin.

The most abundant proteins in chromatin are the *histones*, which are small positively charged proteins of five major types: H1, H2A, H2B, H3, and H4. All histones have a high percentage of arginine and lysine, positively charged amino acids that give the histones a net positive charge. These positive charges attract the negative charges on the phosphates of DNA; this attraction holds the DNA in contact with the histones. A heterogeneous assortment of **nonhistone chromosomal proteins** is also found in eukaryotic chromosomes. At times, variant histones, with somewhat different amino acid sequences, are incorporated into chromatin in place of one of the major histone types. These variants alter chromatin structure and influence its function. For example, some specific variant histones are associated with actively transcribed DNA; these variants are assumed to make the chromatin more open and accessible to the enzymes and proteins that carry out transcription. › **TRY PROBLEM 18**

CONCEPTS

Chromatin, which consists of DNA complexed with proteins, is the material that makes up eukaryotic chromosomes. The most abundant of these proteins are the five types of positively charged histone proteins: H1, H2A, H2B, H3, and H4. Variant histones may at times be incorporated into chromatin in place of the normal histone types.

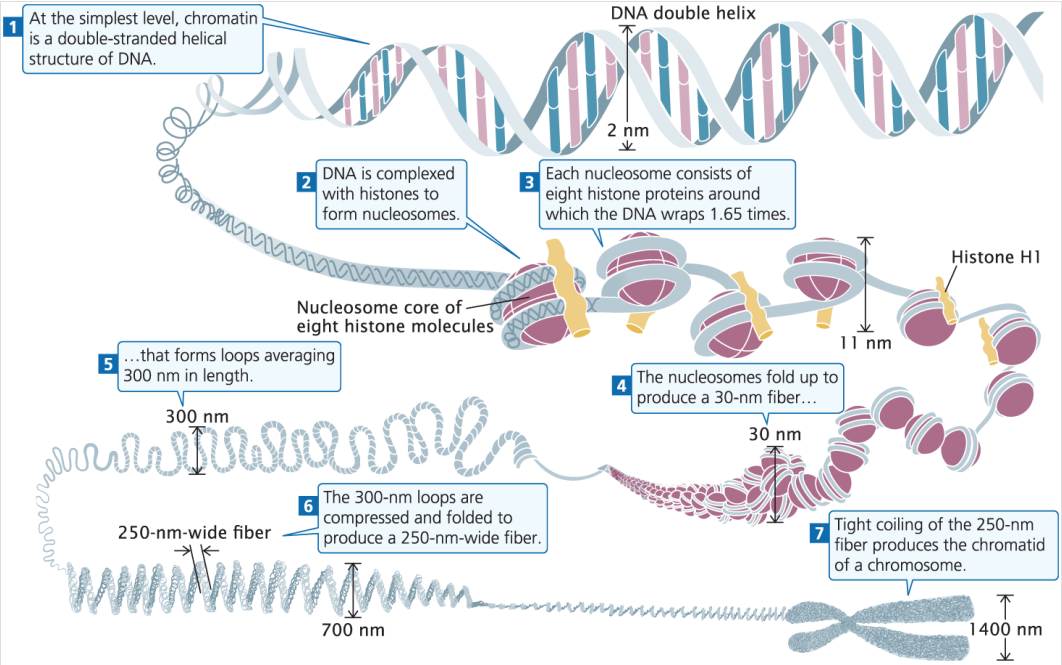
✔ CONCEPT CHECK 3

Neutralizing their positive charges would have which effect on the histone proteins?

a. They would bind the DNA tighter.
b. They would bind less tightly to the DNA.
c. They would no longer be attracted to each other.
d. They would cause supercoiling of the DNA.

THE NUCLEOSOME

Chromatin has a highly complex structure, with several levels of organization (**Figure 11.4**). The simplest level is the double-helical structure of DNA, discussed in Chapter 10. At a more complex level, the DNA molecule is closely associated with proteins and is highly folded to produce a chromosome.



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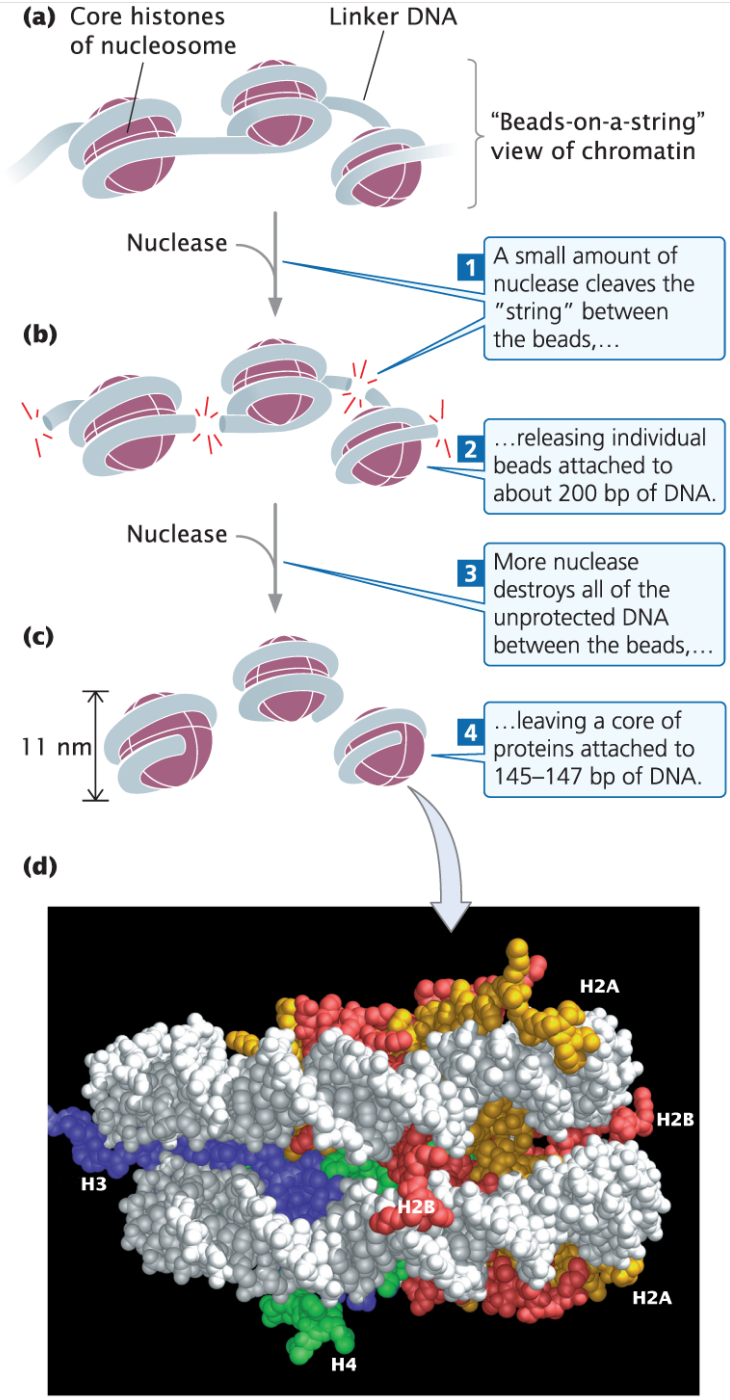
11.4 Chromatin has a highly complex structure, with several levels of organization.

Description

The illustration shows a D N A double helix with a width of 2 n m. A caption reads, at the simplest level, chromatin is a double-stranded helical structure of D N A. The D N A then coils around nucleosome cores that consist of 8 histone molecules. A caption reads, D N A is complexed with histones to form nucleosomes. Each nucleosome consists of eight histone proteins around which the D N A wraps 1 point 6 5 times. The diameter of the D N A-nucleosome complex is 11 n m. Histone 1 protein is bound to the outside of the D N A-nucleosome complex. These nucleosomes fold multiple times and become more and more condensed to eventually form a fiber-like structure. A caption reads, the nucleosomes fold up to produce a 30 n m fiber that forms loops averaging 300 n m in length. The 300 n m loops are then compressed and folded to produce a 250 n m wide fiber. The fiber produced is 700 n m in diameter. This fiber finally produces the chromatids of a chromosome. A caption reads, tight coiling of the 250 n m fiber produces the chromatid of a chromosome. The X shaped chromosome formed has a width of 1400 n m.

When chromatin is isolated from the nucleus of a cell and viewed with an electron microscope, it frequently looks like beads on a string (**Figure 11.5a**). If a small amount of nuclease is added to this structure, the enzyme cleaves the "string" between the "beads," leaving individual beads attached to about 200 bp of DNA (**Figure 11.5b**). If more nuclease is added, the enzyme chews up all of the DNA between the beads and leaves a core of proteins attached to a fragment of DNA (**Figure 11.5c**). Such experiments demonstrate that chromatin is not a random association of proteins and DNA but has a fundamental repeating structure.



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Part d: Reprinted by permission from Macmillan Publishers Ltd. From K. Luger et al., Crystal structure of the nucleosome core particle at 2.8 A resolution, *Nature* 389:251, 1997. ©1997. Courtesy of T. H. Richmond, permission conveyed through Copyright Clearance Center, Inc.

11.5 The nucleosome is the fundamental repeating unit of chromatin. Part *d* shows a space-filling model of the core particle, which consists of two copies each of H2A, H2B, H3, and H4, around which DNA (white) coils.

Description
Illustration a at the top shows a beads-on-a-string view of chromatin where a D N A helix is wound around three nucleosomes with core histones. The D N A portion between two consecutive nucleosomes is labeled as linker D N A. This D N A-nucleosome complex is subjected to a nuclease action. A caption reads, a small amount of nuclease cleaves the string between the beads. Illustration b below shows the linkage D N A is cleaved by the nuclease such that three D N A-nucleosome molecules are formed. A caption reads, releasing individual beads attached to about 200 b p of D N A. More nuclease is added. Illustration c below shows D N A-nucleosome molecules where more unprotected D N A has been destroyed by nuclease. The diameter of these molecules is 11 n m. A caption reads, more nuclease destroys all of the unprotected D N A between the beads, leaving a core of proteins attached to 145 to 147 b p of D N A. Illustration d below shows a space-filling model with 2 copies of each of H 2 A, H 2 B, H 3, and H 4 surrounded by white-colored D N A coils.

The core of protein and DNA produced by digestion with nuclease enzymes is the simplest level of chromatin structure, the **nucleosome** (see Figure 11.4). The nucleosome is a core particle consisting of DNA wrapped about two times around an octamer of eight histone proteins (two copies each of H2A, H2B, H3, and H4), much like thread wound around a spool (**Figure 11.5d**). The DNA in direct contact with the histone octamer is between 145 and 147 bp in length.

Each of the histone proteins that make up the nucleosome core particle has a flexible “tail,” containing from 11 to 37 amino acids,

which extends out from the nucleosome. Positively charged amino acids in the tails of the histones interact with the negative charges of the phosphates on the DNA, keeping the DNA and histones tightly associated. The tails of one nucleosome may also interact with neighboring nucleosomes, which facilitates compaction of the nucleosomes themselves. Chemical modifications of the histone tails bring about changes in chromatin structure (discussed in the next section) that are necessary for gene expression.

The fifth type of histone, H1, is not a part of the nucleosome core particle but plays an important role in nucleosome structure. H1 binds to 20–22 bp of DNA where the DNA joins and leaves the histone octamer (see Figure 11.4) and helps lock the DNA into place, acting as a clamp around the nucleosome.

Each nucleosome encompasses about 167 bp of DNA. Nucleosomes are located at regular intervals along the DNA molecule and are separated from one another by **linker DNA**, which varies in size among cell types; in most cells, linker DNA comprises from about 30 to 40 bp. Nonhistone chromosomal proteins may be associated with this linker DNA, and a few also appear to bind directly to the core particle.

Although histone proteins are not present in bacteria, archaea have two types of histones that are similar to the H3 and H4 histones found in eukaryotes. These archaeal histones associate in groups of four, forming a tetramer instead of the octamers seen in eukaryotes.

The DNA of archaea wraps around the tetramers of histones to form structures similar to the nucleosomes of eukaryotes, each encompassing about 60 bp of DNA. The nucleosomes of archaea occupy specific positions along the DNA, much as they do in eukaryotes, which suggests that they may play a role in gene expression (see Chapter 17). ❯ **TRY PROBLEMS 19 AND 22**

HIGHER-ORDER CHROMATIN STRUCTURE

When chromatin is in a condensed form, nucleosomes fold on themselves to form a dense, tightly packed structure (see Figure 11.4) that makes up a fiber with a diameter of about 30 nm. Some recent research suggests that the 30-nm fiber consists of two interwound stacks of nucleosomes, with the linker DNA between successive nucleosomes crisscrossing back and forth in the interior of the fiber. However, other structures of the 30-nm fiber have also been proposed, and indeed, different structures may exist under different conditions within the nucleus.

The precise structure of chromatin above the level of the 30-nm fiber is poorly understood. The next level of chromatin structure is a series of loops of the 30-nm fiber (see Figure 11.4), each anchored at its base by proteins. On average, each loop encompasses some 20,000 to 100,000 bp of DNA and is about 300 nm in length, but the individual loops vary considerably. The 300-nm loops are packed and folded to produce a 250-nm-wide fiber. Tight helical coiling of the 250-nm fiber, in turn, produces the structure that appears in

metaphase: an individual chromatid approximately 700 nm in width. You can view the different levels of chromatin structure in **Animation 11.1**.

The traditional view of chromosome structure has been that nucleosomes and their packing arrangement lead to higher-order chromosome structure. However, some research has challenged this view. Researchers have reported that chromosomes can form even in the absence of nucleosomes, although chromosomes without nucleosomes were wider and more fragile than chromosomes with nucleosomes. The research indicates that condensins, molecules that are involved in chromosome formation during mitosis (see Chapter 2), are essential for higher-order chromosome structure. Nucleosomes are also important, but their primary role may be in DNA compaction and protection more than shaping the chromosome. How condensins might shape chromosomes is still being studied.

During interphase, different chromosomes and different parts of the same chromosome do not associate randomly within the nucleus. Instead, large regions of chromatin form spatially interacting regions called **topologically associated domains (TADs)**. These domains may encompass tens of thousands to millions of bp of DNA. Within a TAD, regions of chromatin lie in close proximity and interact with one another but are separated in space from other TADs. TADs are a consistent and important feature of eukaryotic genomes; they contribute to the control of gene expression by bringing genes and

distant regulatory elements (called enhancers, see Chapter 17) into close proximity.

CONCEPTS

The nucleosome consists of a core particle of eight histone proteins and the DNA that wraps around them. A single H1 histone associates with each core particle. Nucleosomes are separated by linker DNA. Nucleosomes fold to form a 30-nm chromatin fiber, which appears as a series of loops that pack to create a 250-nm fiber. Helical coiling of the 250-nm fiber produces a chromatid. Larger areas of chromatin form interacting regions called topologically associated domains.

✔ CONCEPT CHECK 4

How many copies of the H2B histone would be found in chromatin containing 50 nucleosomes?

a. 5
b. 10
c. 50
d. 100

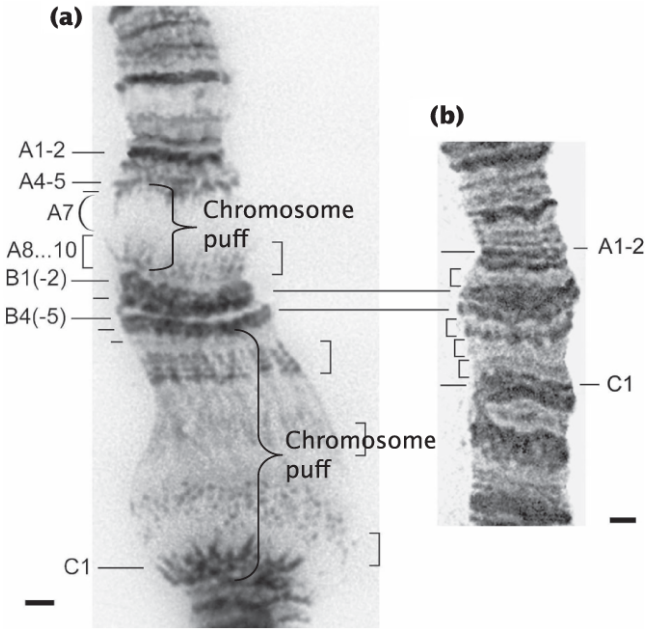
Changes in Chromatin Structure

Although eukaryotic DNA must be tightly packed to fit into the cell nucleus, it must also periodically unwind to undergo transcription and replication. Polytene chromosomes and DNase I sensitivity, discussed in the following sections, are visible evidence of the dynamic nature of chromatin structure.

POLYTENE CHROMOSOMES

Giant chromosomes called **polytene chromosomes** are found in certain tissues of *Drosophila* larvae and some other organisms (Figure 11.6). Polytene chromosomes have provided researchers with evidence of the changing nature of chromatin structure. These large, unusual chromosomes arise when repeated rounds of DNA replication take place without accompanying cell divisions, producing thousands of copies of DNA that lie side by side. When polytene chromosomes are stained with dyes, numerous bands are revealed. Under certain conditions, the bands may exhibit **chromosome puffs**—localized swellings of the chromosome. Each puff is a region of the chromatin that has a relaxed structure and, consequently, a more open state. Research indicates that chromosome puffs are regions of active transcription. This correlation between the occurrence of transcription and the relaxation of chromatin at a puff site indicates that chromatin structure undergoes dynamic change associated with gene activity.

THINK-PAIR-SHARE Question 2



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Courtesy of Dmitri Novikov.

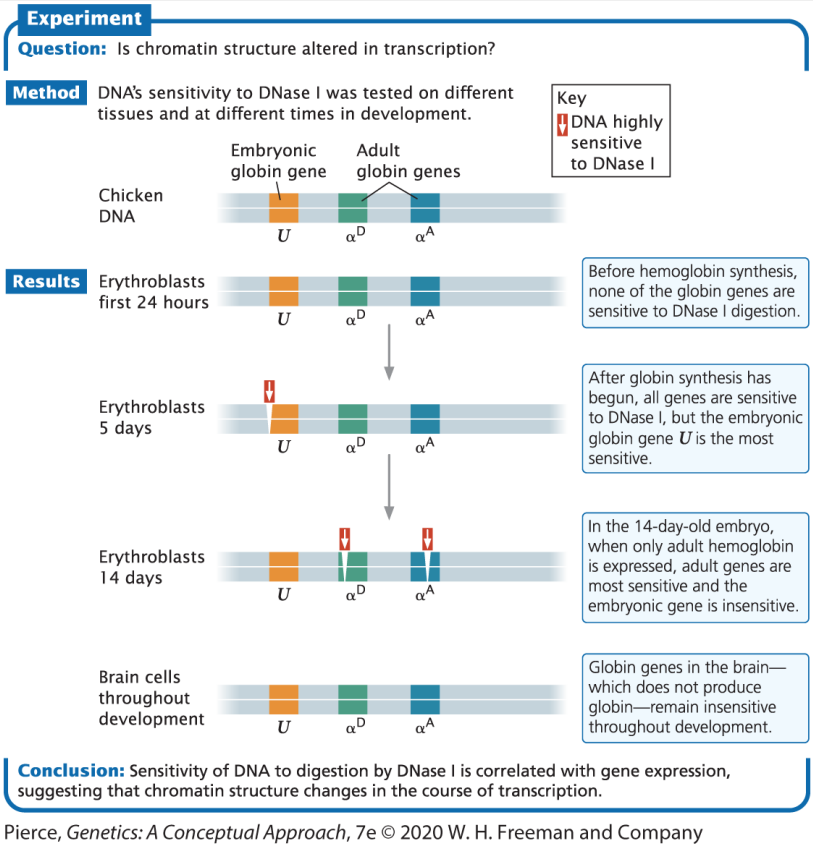
11.6 Chromosome puffs are regions of relaxed chromatin where active transcription is taking place. (a) Chromosome puffs on giant polytene chromosomes isolated from the salivary glands of larval *Drosophila*. (b) The corresponding region without chromosome puffs. The density of the stain correlates with the density of the chromatin.

Description
Illustration a at the left shows a giant polytene chromosomes with a chromosome puff between A 7, A 8, to 10 and another between B 4 (negative 5) to C 1. There are multiple dark bands while the chromosome puffs are light colored regions. Illustration b at the right shows the same chromosome compacted and without the chromosome puffs.

DNASE I SENSITIVITY

A second piece of evidence indicating that chromatin structure changes with gene activity is sensitivity to DNase I, an enzyme that

digests DNA. The ability of this enzyme to digest DNA depends on chromatin structure: when DNA is tightly bound to histone proteins, it is less sensitive to DNase I, whereas unbound DNA is more sensitive to DNase I. The results of experiments that examined the effect of DNase I on specific globin genes in chick embryos showed that DNase sensitivity is correlated with gene activity. Globin genes encode several types of hemoglobin, which are expressed in the erythroblasts (precursors of red blood cells) of chickens at different stages of development (Figure 11.7). These types of experiments demonstrate that transcriptionally active genes are sensitive to DNase I, indicating that the chromatin structure is more exposed during transcription.



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11.7 DNase I sensitivity is correlated with the transcription of globin genes in chick embryos. The *U* gene encodes embryonic hemoglobin; the α^{D} and α^{A} genes encode adult hemoglobin. The red arrows indicate sites of DNase I digestion.

Description
The illustration begins with a question that reads, Is chromatin structure altered in transcription? The illustration shows methods below where a chicken D N A has the following three genes: embryonic globin gene (U), adult globin genes (alpha superscript D and alpha superscript A). The results below show the following: Erythroblasts after first 24 hours show no sensitivity to DNase 1. Text reads, before hemoglobin synthesis, none of the globin genes are sensitive to DNase 1 digestion. Erythroblasts after 5 days show increased DNase 1 sensitivity at the beginning of embryonic globin gene (U). Text reads, after globin synthesis has begun, all genes are sensitive to DNase 1, but the embryonic globin gene U is the most sensitive. Erythroblasts after 14 days show increased DNase 1 sensitivity at the beginning of adult globin gene alpha superscript D and in the center of alpha superscript A. Text reads, in the 14-day old embryo, when only adult hemoglobin is expressed, adult genes are most sensitive and the embryonic gene is insensitive. Brain cells throughout development are not sensitive to DNase 1. Text reads, globin genes in the brain, which does not produce globin, remain insensitive throughout development. A caption below reads, Conclusion: Sensitivity of D N A to digestion by DNase 1 is correlated with gene expression, suggesting that chromatin structure changes in the course of transcription.

What is the nature of the change in chromatin structure that produces chromosome puffs and DNase I sensitivity? In both cases, the chromatin relaxes; presumably, the histones loosen their grip on the DNA. One process that alters chromatin structure is acetylation. Enzymes called acetyltransferases attach acetyl groups to lysine amino acids on the histone tails. This modification reduces the positive charges that normally exist on lysine and destabilizes the nucleosome structure, so the histones hold the DNA less tightly.

Other chemical modifications of the histone proteins, such as methylation and phosphorylation, also alter chromatin structure, as do special chromatin-remodeling proteins that bind to the DNA. ❯

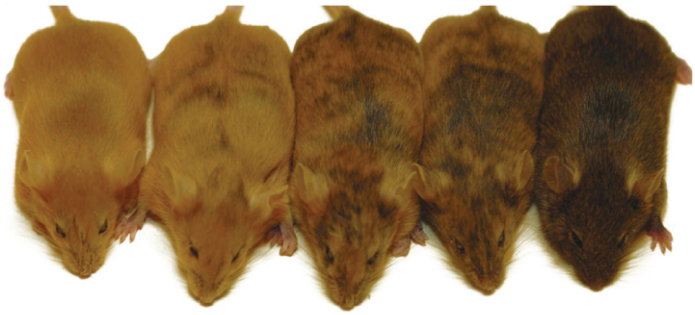
TRY PROBLEM 21

EPIGENETIC CHANGES ASSOCIATED WITH CHROMATIN MODIFICATIONS

We have now seen how chromatin structure can be altered by chemical modification of the histone proteins. A number of other changes can also affect chromatin structure, including the methylation of DNA (see Chapter 10), the use of variant histone proteins in the nucleosome, and the binding of nonhistone proteins to DNA and chromatin. Although these changes do not alter the DNA sequence, they often have major effects on the expression of genes, which will be discussed in more detail in Chapter 17.

Some changes in chromatin structure are retained through cell division, so that they are passed on to future generations of cells and even occasionally to future generations of organisms. Stable alterations of chromatin structure that may be passed on to descendant cells or individuals are frequently referred to as **epigenetic changes,** or simply *epigenetics* (see Chapter 5). For example, the *agouti* locus helps determine coat color in mice: parents that have identical DNA sequences but have different degrees of methylation on their DNA may give rise to offspring with

different coat colors (**Figure 11.8**). Such epigenetic changes have been observed in a number of organisms and are responsible for a variety of phenotypic effects. Unlike mutations, epigenetic changes do not alter the DNA sequence, are capable of being reversed, and are often influenced by environmental factors (see Chapter 21 for more details).



Cropley et al., 2006, Germ-line epigenetic modification of the murine A^{vy} allele by nutritional supplementation, *PNAS* November 14, 2006 vol. 103, no. 46 pp. 7308–17312. ©2006 National Academy of Sciences, U.S.A.

11.8 Variation in DNA methylation at the *agouti* locus produces different coat colors in mice.

CONCEPTS

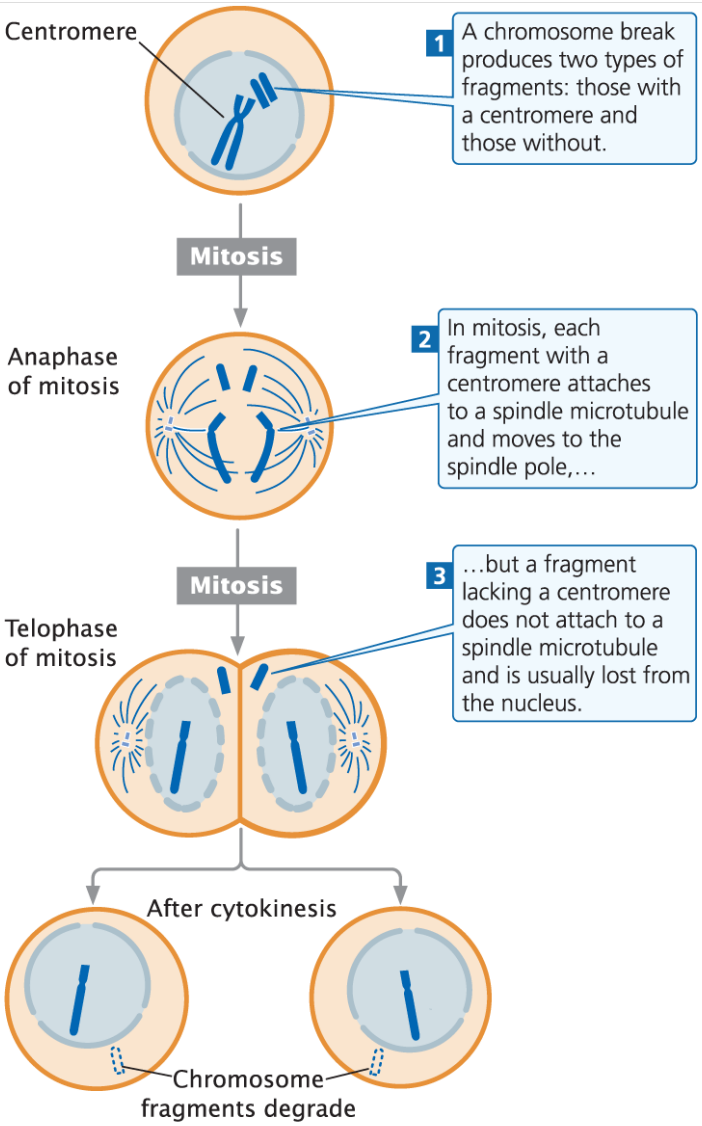
Epigenetic changes are alterations of chromatin or DNA structure that do not include changes in the base sequence but are stable and passed on to descendant cells or organisms. Some epigenetic changes result from alterations of chromatin structure.

11.2 Eukaryotic Chromosomes Possess Centromeres and Telomeres

Chromosomes, as we have seen, segregate in mitosis and meiosis and remain stable over many cell divisions. These properties of chromosomes arise in part from special structural features, including centromeres and telomeres.

Centromere Structure

The centromere, a constricted region of the chromosome, is the attachment site for the kinetochore and for spindle microtubules; this chromosome structure is necessary for proper chromosome movement in mitosis and meiosis (see Figure 2.7). The essential role of the centromere in chromosome movement was recognized by early geneticists, who observed the consequences of chromosome breakage. When a chromosome break produces two fragments, one with a centromere and one without, the chromosome fragment containing the centromere attaches to a spindle microtubule and moves to the spindle pole. The fragment lacking a centromere fails to connect to a spindle microtubule and is usually lost because it fails to move into the nucleus of a daughter cell during mitosis (**Figure 11.9**).



11.9 Chromosome fragments that lack centromeres are lost in mitosis.

Description

The illustration shows a cell with two fragments of chromosomes inside the nucleus. One of the fragments has a centromere while another has no centromere. A caption reads, a chromosome break produces 2 types of fragments, those with a centromere and those without. Mitosis occurs and the illustration shows anaphase of mitosis below where each fragment with centromere moves towards one of the cell poles after attaching to the spindle fiber. A caption reads, in mitosis, each fragment with a centromere attaches to a

spindle microtubule and moves to the spindle pole. Mitosis progresses and telophase below shows the cell membrane dividing into two compartments. A nuclear envelope starts developing around fragments with centromere, in both compartments. The other fragments without centromere remain outside the nuclear membranes. A caption reads, but a fragment lacking a centromere does not attach to a spindle microtubule and is usually lost from the nucleus. Cytokinesis occurs and two daughter cells are formed below. The fragment without a centromere degrades in both the daughter cells.

What are the key features of the centromere? In *Drosophila*, *Arabidopsis*, and humans, centromeres span hundreds of thousands of base pairs. Most of the centromere is made up of heterochromatin. Surprisingly, there are no specific sequences that are found in all centromeres, which raises the question of what exactly determines where the centromere is. Research suggests that most centromeres are not defined by DNA sequence but rather by changes in chromatin structure, referred to as epigenetic modifications (see Chapter 21). Nucleosomes in the centromeres of most eukaryotes have a variant histone protein called CENP-A (also referred to as CenH3), which takes the place of the usual H3 histone. The CENP-A variant histone is required for the assembly of proteins associated with the kinetochore; the presence of the CENP-A histone probably alters the nucleosome and chromatin structure, allowing kinetochore proteins to bind and spindle microtubules to attach.

CONCEPTS

The centromere is a region of the chromosome to which spindle microtubules attach. Centromeres display considerable variation in their DNA sequences and are distinguished by

epigenetic alterations to chromatin structure, including the use of a variant H3 histone in the nucleosome.

✔ CONCEPT CHECK 5

What happens to a chromosome that loses its centromere?

Telomere Structure

Telomeres are the natural ends of a chromosome (see Figure 2.7 and the introduction to this chapter). Pioneering work by Hermann Muller (in fruit flies) and Barbara McClintock (in corn) showed that chromosome breaks produce unstable ends that have a tendency to stick together and enable the chromosome to be degraded. Because attachment and degradation do not happen to the ends of a chromosome that has telomeres, the telomeres must serve as caps that stabilize the chromosome. Telomeres also provide a means of replicating the ends of a chromosome, as we will see in Chapter 12. In 2009, Elizabeth Blackburn, Carol Greider, and Jack Szostak were awarded the Nobel Prize in physiology or medicine for discovering the structure of telomeres and how they are replicated.

Telomeres have now been isolated from protozoans, plants, humans, and other organisms; most are similar in structure (**Table 11.2**). These **telomeric sequences** usually consist of repeated units of a series of adenine or thymine nucleotides followed by several guanine

nucleotides, taking the form 5′–(A or T)$_m$G$_n$–3′, where *m* ranges from 1 to 4 and *n* is 2 or more. For example, the repeating unit in human telomeres is 5′–TTAGGG–3′, which may be repeated from hundreds to thousands of times. The sequence is always oriented with the string of Gs and Cs toward the end of the chromosome, as shown here:

Toward centromere ← 5′–TTAGGGTTAGGGTTAGGG–3′ / 3′–AATCCCAATCCCAATCCC–5′ → end of chromosome

Description
The illustration shows two complementary strands. The first strand begins from 5 prime and the sequence is as follows: T; T; A; G; G; G; T; T; A; G; G; G; T; T; A; G; G; G. It ends at 3 prime. The second strand begins at 3 prime and the sequence is as follows: A; A; T; C; C; C; A; A; T; C; C; C; A; A; T; C; C; C. It ends at 5 prime. Text at the beginning of both reads, towards centromere, and text at the end of both reads, end of the chromosome.

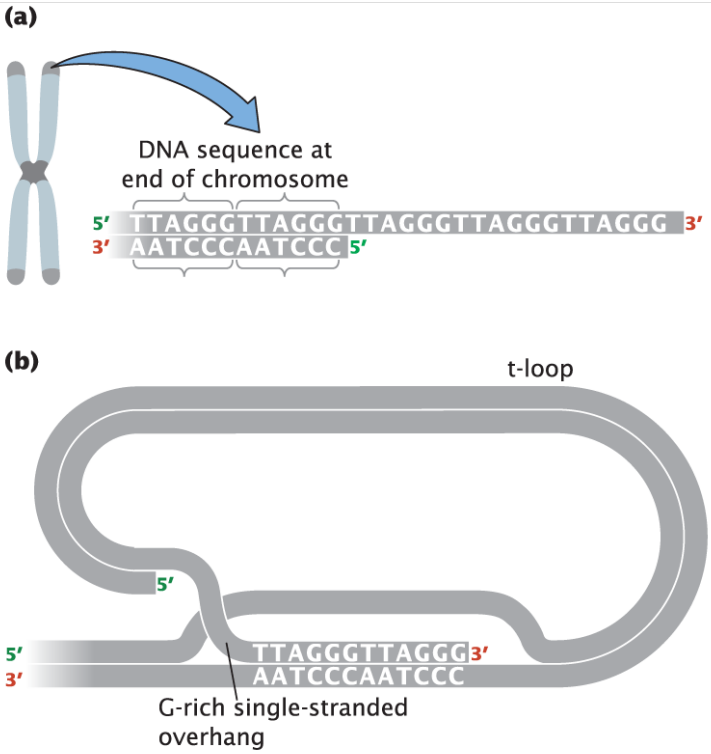
TABLE 11.2 DNA sequences typically found in telomeres of various organisms

Organism	Sequence
Tetrahymena (protozoan)	5′–TTGGGG–3′ 3′–AACCCC–5′
Saccharomyces (yeast)	5′–T1–6GTG2–3–3′ 3′–A1–6CAC2–3–5′
Caenorhabditis (nematode)	5′–TTAGGC–3′ 3′–AATCCG–5′

Vertebrate	5′–TTAGGG–3′ 3′–AATCCC–5′
Arabidopsis (plant)	5′–TTTAGGG–3′ 3′–AAATCCC–5′

Data from: V. A. Zakian, *Science* 270:1602, 1995.

The G-rich strand often protrudes beyond the complementary C-rich strand at the end of the chromosome (**Figure 11.10a**), in which case it is called the G-rich 3′ overhang. The G-rich 3′ overhang in the telomeres of mammals is from 50 to 500 nucleotides long. Special proteins bind to this G-rich single-stranded sequence, protecting the telomere from degradation and preventing the ends of chromosomes from sticking together. A multiprotein complex called **shelterin** binds to telomeres and protects the ends of the DNA from being inadvertently repaired as a double-stranded break in the DNA. In some cells, the G-rich 3′ overhang may fold over and pair with a short stretch of DNA to form a structure called a t-loop, which also functions in protecting the end of the telomere from degradation (**Figure 11.10b**).



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11.10 DNA at the ends of eukaryotic chromosomes consists of telomeric sequences. (a) The G-rich strand at the telomere is longer than the C-rich strand. (b) In some cells, the G-rich strand folds over and pairs with a short stretch of DNA to form a t-loop.

Description

Illustration “a” at the top shows an X-shaped chromosome with the telomeres in gray and an arrow pointing from the telomere to the D N A telomeric sequence. The 5 prime to 3 prime strand extends 18 nucleotides out from end of the other strand. The repeating sequence contains base pairs T T A G G G. Illustration “b” below shows the T T A G G G sequence pairing with the complementary sequence at an earlier part of the telomere and dislocating the D N A that was previously bound.

Because DNA polymerases (enzymes that synthesize DNA) cannot replicate the ends of chromosomes, telomeres in most somatic cells

shorten each time the cell divides (see section on Replication at the Ends of Chromosomes in Chapter 12). Single-cell eukaryotes, germ cells, and early embryonic cells produce an enzyme called telomerase, which lengthens telomeres and prevents chromosome shortening (see Chapter 12). Somatic cells have additional mechanisms that regulate the length of telomeres by trimming and elongating telomeric sequences.

CONCEPTS

A telomere is the stabilizing end of a chromosome. At the end of each telomere are many short telomeric sequences.

✔ CONCEPT CHECK 6

Which of the following is a characteristic of DNA sequences at the telomeres?

a. One strand consists of guanine and adenine (or thymine) nucleotides.
b. They consist of repeated sequences.
c. One strand protrudes beyond the other, creating some single-stranded DNA at the end.
d. All of the above

11.3 Eukaryotic DNA Contains Several Classes of Sequence Variation

Eukaryotic organisms vary dramatically in the amount of DNA per cell, a quantity termed an organism's **C-value** (Table 11.3). Each cell of a fruit fly, for example, contains about 35 times the amount of DNA found in a cell of the bacterium *E. coli*. In general, eukaryotic cells contain more DNA than prokaryotic cells do, but variation among eukaryotes in their C-values is huge. Human cells contain more than 10 times the amount of DNA found in *Drosophila* cells, whereas some salamander cells contain 20 times as much DNA as human cells. Clearly, these differences in C-value cannot be explained simply by differences in organismal complexity. So what is all of the extra DNA in eukaryotic cells doing? This question has been termed the **C-value paradox**. We do not yet have a complete answer to the C-value paradox, but analysis of eukaryotic DNA sequences has revealed a complexity that is absent from prokaryotic DNA.

TABLE 11.3 Genome sizes of various organisms

Organism	Approximate Genome Size (bp)
λ (bacteriophage)	50,000

Escherichia coli (bacterium)	4,640,000
Saccharomyces cerevisiae (yeast)	12,000,000
Arabidopsis thaliana (plant)	125,000,000
Drosophila melanogaster (insect)	170,000,000
Homo sapiens (human)	3,200,000,000
Zea mays (corn)	4,500,000,000
Amphiuma (salamander)	765,000,000,000

The Denaturation and Renaturation of DNA

The first clue that eukaryotic DNA contains several types of sequences not present in prokaryotic DNA came from studies in which double-stranded DNA was separated and then allowed to reassociate. When double-stranded DNA in solution is heated, the hydrogen bonds that hold the two nucleotide strands together are weakened, and with enough heat, the two strands separate completely, a process called **denaturation** or *melting*. The

temperature at which DNA denatures, called the **melting temperature (T_m)**, depends on the base sequence of the particular sample of DNA: G–C base pairs have three hydrogen bonds, whereas A–T base pairs have only two, so the separation of G–C pairs requires more heat (energy) than does the separation of A–T pairs.

The denaturation of DNA by heating is reversible: if single-stranded DNA is slowly cooled, single strands will collide and hydrogen bonds will form again between complementary base pairs, producing double-stranded DNA. This reaction is called **renaturation** or *reannealing*.

Two single-stranded molecules of DNA from different sources, such as different organisms, will anneal if they are complementary; this process is termed **hybridization**. For hybridization to take place, the two strands from the different sources do not have to be complementary at all their bases—just at enough bases to hold the two strands together. The extent of hybridization between DNA from two species can be used to measure the similarity of their nucleic acid sequences and to assess their evolutionary relationship. The rate at which hybridization takes place also provides information about the sequence complexity of DNA. **› TRY PROBLEM 27**

Types of DNA Sequences in Eukaryotes

Eukaryotic DNA consists of at least three classes of sequences: unique-sequence DNA, moderately repetitive DNA, and highly repetitive DNA. **Unique-sequence DNA** consists of sequences that are present only once or, at most, a few times in the genome. This DNA includes sequences that encode proteins, as well as a great deal of DNA whose function is unknown. Genes that are present in a single copy constitute roughly 25% to 50% of the protein-encoding genes in most multicellular eukaryotes. Other genes within unique-sequence DNA are present in several similar but not identical copies and are collectively referred to as a **gene family**. Most gene families arose through duplication of an existing gene and include just a few member genes, but some, such as those that encode immunoglobulin proteins in vertebrates, contain hundreds of members. The genes that encode β-like globins are another example of a gene family. In humans, there are six β-globin genes, clustered together on chromosome 11. The polypeptides encoded by these genes join with α-globin polypeptides to form hemoglobin molecules, which transport oxygen in the blood.

Other sequences, called **repetitive DNA**, exist in many copies. Some eukaryotic organisms have large amounts of repetitive DNA; for example, almost half of the human genome consists of repetitive DNA. A major class of repetitive DNA is **moderately repetitive DNA**,

which typically consists of sequences from 150 to 300 bp in length (although they may be longer) that are repeated many thousands of times. Some of these sequences perform important functions for the cell; for example, multiple copies of the genes for ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) make up a part of the moderately repetitive DNA. However, the function of much moderately repetitive DNA is unknown, and indeed, it may have no function.

Moderately repetitive DNA itself can be divided into two types of repeats. **Tandem repeats** appear one after another and tend to be clustered at particular locations on the chromosomes. **Interspersed repeats** are scattered throughout the genome. An example of an interspersed repeat is the *Alu* sequence, an approximately 300-bp sequence that is present more than a million times and constitutes 11% of the human genome, although it has no obvious cellular function. Short repeats such as the *Alu* sequence are called **SINEs (short interspersed elements)**. Longer interspersed repeats consisting of several thousand base pairs are called **LINEs (long interspersed elements)**. One class of LINEs, called LINE1, constitutes about 17% of the human genome. Most interspersed repeats are the remnants of transposable elements, sequences that can multiply and move (see Chapter 18).

The other major class of repetitive DNA is **highly repetitive DNA**. These short sequences, often less than 10 bp in length, are present

in hundreds of thousands to millions of copies that are repeated in tandem and clustered in certain regions of the chromosome, especially in centromeres and telomeres. Highly repetitive DNA is sometimes called *satellite DNA* because its proportions of the four bases differ from those of other DNA sequences, and therefore it separates as a satellite fraction when centrifuged at high speeds in a density gradient (see Figure 12.2). Highly repetitive DNA is rarely transcribed into RNA. Although it may contribute to centromere and telomere function, most highly repetitive DNA has no known function.

Organization of Genetic Information in Eukaryotes

DNA-hybridization reactions and, more recently, direct sequencing of eukaryotic genomes have not only revealed the types of sequences we have just described but also told us a lot about how genetic information is organized within chromosomes. We now know that the density of genes varies greatly among and within chromosomes. For example, human chromosome 19 has a high density of genes, with about 26 genes per million base pairs. Chromosome 13, on the other hand, has only about 6.5 genes per million base pairs. Gene density can also vary among different regions of the same chromosome: some parts of the long arm of chromosome 13 have only 3 genes per million base pairs, whereas

other parts have almost 30 genes per million base pairs. And the short arm of chromosome 13 contains almost no genes, consisting entirely of heterochromatin.

CONCEPTS

Eukaryotic DNA comprises three major classes: unique-sequence DNA, moderately repetitive DNA, and highly repetitive DNA. Unique-sequence DNA consists of sequences that exist in one or a few copies; moderately repetitive DNA consists of sequences that may be several hundred base pairs in length and are present in thousands to hundreds of thousands of copies. Highly repetitive DNA consists of very short sequences repeated in tandem and is present in hundreds of thousands to millions of copies. The density of genes varies greatly among and even within chromosomes.

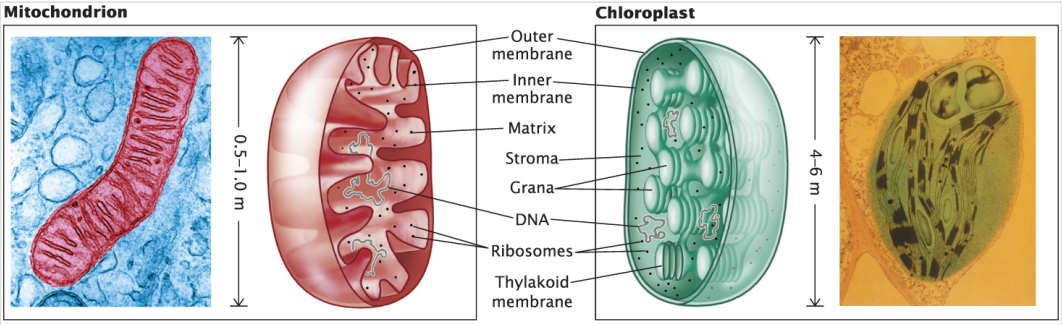
✔ CONCEPT CHECK 7

Most of the genes that encode proteins are found in

a. unique-sequence DNA.
b. moderately repetitive DNA.
c. highly repetitive DNA.
d. All of the above

11.4 Organelle DNA Has Unique Characteristics

As we have seen, eukaryotic chromosomes reside within the nucleus and have a complex structure consisting of DNA and associated histone proteins. However, some DNA found in eukaryotic cells occurs outside the nucleus, has a very different organization, and exhibits a different pattern of inheritance from nuclear DNA. This DNA occurs in mitochondria and chloroplasts, which are membrane-bounded organelles located in the cytoplasm of eukaryotic cells (Figure 11.11).



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Left: Don W. Fawcett/Science Source. Right: Biophoto Associates/Science Source.

11.11 Comparison of the structures of mitochondria and chloroplasts.

Description

The electron micrograph on the left shows red-colored mitochondria against blue background with randomly present circular structures. The diagram on the left shows all the parts of mitochondria. The outermost layer is labeled as outer membrane. The inner membrane is folded inside forming zig-zag pattern. The inside of the inner membrane is labeled as matrix. The matrix has a large number of small, round structures spread throughout and labeled as ribosomes. Two D N A strands are present in the center and

bottom of the mitochondria respectively and are nearly arranged in a circular manner. Its length is 0 point 5 to 1 point 0 m. The illustration on the right shows a chloroplast. The outermost layer is labeled as outer membrane and a layer inside is labeled as inner membrane. The inside of the chloroplast is labeled as stroma. Circular disc shaped structures are stacked and are labeled as grana. The layer covering the circular disc shaped structures is labeled as thylakoid membrane. D N A strands are present randomly in the mitochondria and are nearly arranged in a circular manner. Its length is 4 to 6 m. The electron micrograph on the right shows green-colored chloroplast against yellow background.

Mitochondrion and Chloroplast Structure

Mitochondria are present in almost all eukaryotic cells, whereas chloroplasts are found in plants, algae, and some protists. Both organelles generate ATP, the universal energy carrier of cells.

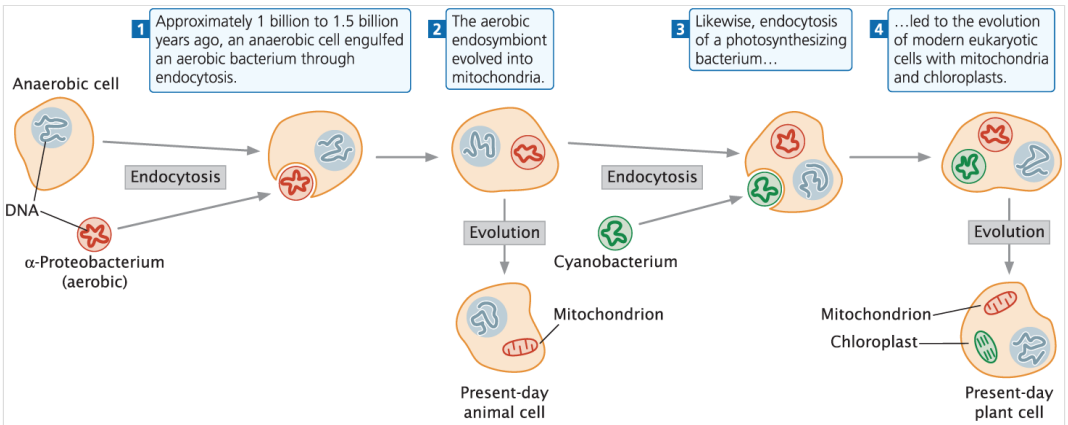
Mitochondria are tubular structures that are from 0.5 to 1.0 micrometer (μm) in diameter, about the size of a typical bacterium, whereas chloroplasts are typically from about 4 to 6 μm in diameter. Both are surrounded by two membranes enclosing a region (called the matrix in mitochondria and the stroma in chloroplasts) that contains enzymes, ribosomes, RNA, and DNA. In mitochondria, the inner membrane is highly folded; embedded within it are the enzymes that catalyze electron transport and oxidative phosphorylation. Chloroplasts have a thylakoid membrane, which is highly folded and stacked to form aggregates called grana. This

membrane bears the pigments and enzymes required for photophosphorylation. New mitochondria and chloroplasts arise by the division of existing organelles; these divisions take place throughout the cell cycle and are independent of mitosis and meiosis.

Mitochondria and chloroplasts possess DNA that encodes some polypeptides used by the organelles, as well as rRNAs found in the ribosomes and the tRNAs needed for the translation of these proteins. The genes for most of the 900 or so structural proteins and enzymes found in mitochondria, however, are actually encoded by nuclear DNA; the mitochondrial genome typically encodes only a few proteins and a few rRNA and tRNA molecules needed for mitochondrial protein synthesis.

The Endosymbiotic Theory

Chloroplasts and mitochondria are similar to bacteria in many ways. This resemblance is not superficial; indeed, there is compelling evidence that these organelles evolved from bacteria. The **endosymbiotic theory** (**Figure 11.12**) proposes that mitochondria and chloroplasts were once free-living bacteria that became internal inhabitants (endosymbionts) of early eukaryotic cells. It is assumed that over evolutionary time, many of the endosymbionts' original genes were subsequently lost (because nuclear genes existed that provided the same function) or were transferred to the nucleus.



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11.12 The endosymbiotic theory proposes that mitochondria and chloroplasts in eukaryotic cells arose from bacteria.

Description

The illustration shows an anaerobic cell on the left with D N A strand in its nucleus and an alpha proteobacterium (aerobic) with a nearly star-shaped D N A. Endocytosis occurs and the anaerobic cells engulf aerobic bacterium. Text reads, approximately 1 billion to 1.5 billion years ago, an anaerobic cell engulfed an aerobic bacterium through endocytosis. The cell which engulfed the bacterium undergoes evolution and produces the present-day animal cell with a mitochondrion. Text reads, the aerobic endosymbiont evolved into mitochondria. The cell which engulfed bacterium also engulfs a cyanobacterium with almost circular D N A strand, through endocytosis. Evolution occurs and the present day plant cell is produced below. The plant cell has a chloroplast and mitochondria. Text reads, likewise, endocytosis of a photosynthesizing bacterium led to the evolution of modern eukaryotic cells with mitochondria and chloroplasts.

A great deal of evidence supports the idea that mitochondria and chloroplasts originated as bacterial cells. Many modern single-celled eukaryotes (protists) are hosts to endosymbiotic bacteria. Mitochondria and chloroplasts are similar in size to present-day bacteria and possess their own DNA, which shares many characteristics with bacterial DNA. Mitochondria and chloroplasts

possess ribosomes, some of which are similar in size and structure to bacterial ribosomes. In addition, antibiotics that inhibit protein synthesis in bacteria but do not affect protein synthesis in eukaryotic cells do inhibit protein synthesis in these organelles.

The strongest evidence for the endosymbiotic theory comes from studies of DNA sequences, which demonstrate that sequences in mtDNA and cpDNA are more closely related to sequences in the genes of bacteria than they are to those found in the eukaryotic nucleus. All of this evidence indicates that mitochondria and chloroplasts are more closely related to bacterial cells than they are to the eukaryotic cells in which they are now found.

CONCEPTS

Mitochondria and chloroplasts are membrane-bounded organelles of eukaryotic cells that generally possess their own DNA. The well-supported endosymbiotic theory proposes that these organelles began as free-living bacteria that developed stable endosymbiotic relations with early eukaryotic cells.

✔ CONCEPT CHECK 8

What evidence supports the endosymbiotic theory?

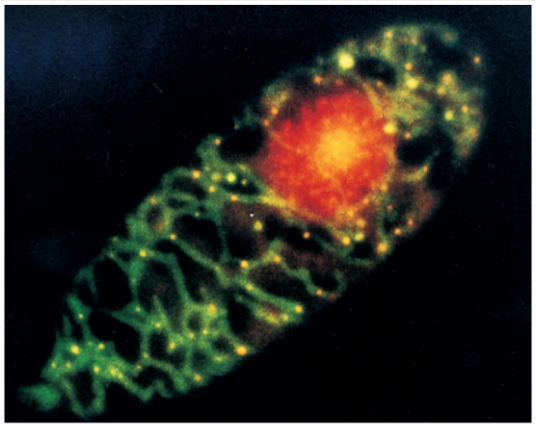
Uniparental Inheritance of Organelle-Encoded Traits

Mitochondria and chloroplasts are present in the cytoplasm and are usually inherited from a single parent. Thus, traits encoded by mtDNA and cpDNA exhibit uniparental inheritance (see Chapter 5). In animals, mtDNA is inherited almost exclusively from the female parent, although occasional male transmission of mtDNA has been documented. In 2018, researchers demonstrated that paternal transmission of mtDNA also occasionally occurs in humans, although it appears to be very rare. Maternal inheritance of animal mtDNA may be partly a function of gamete size: sperm are much smaller than eggs and hold fewer mitochondria. However, in many animals, paternal mitochondria are selectively eliminated by autophagy, a process in which mitochondria are digested by the cell. In these cases, paternal mitochondria are marked for destruction, whereas maternal mitochondria are not; the mechanism that produces this difference is not known. Paternal inheritance of chloroplast DNA is common in gymnosperms (conifers) and in a few angiosperms (flowering plants). Some plants even exhibit biparental inheritance of mtDNA and cpDNA.

REPLICATIVE SEGREGATION

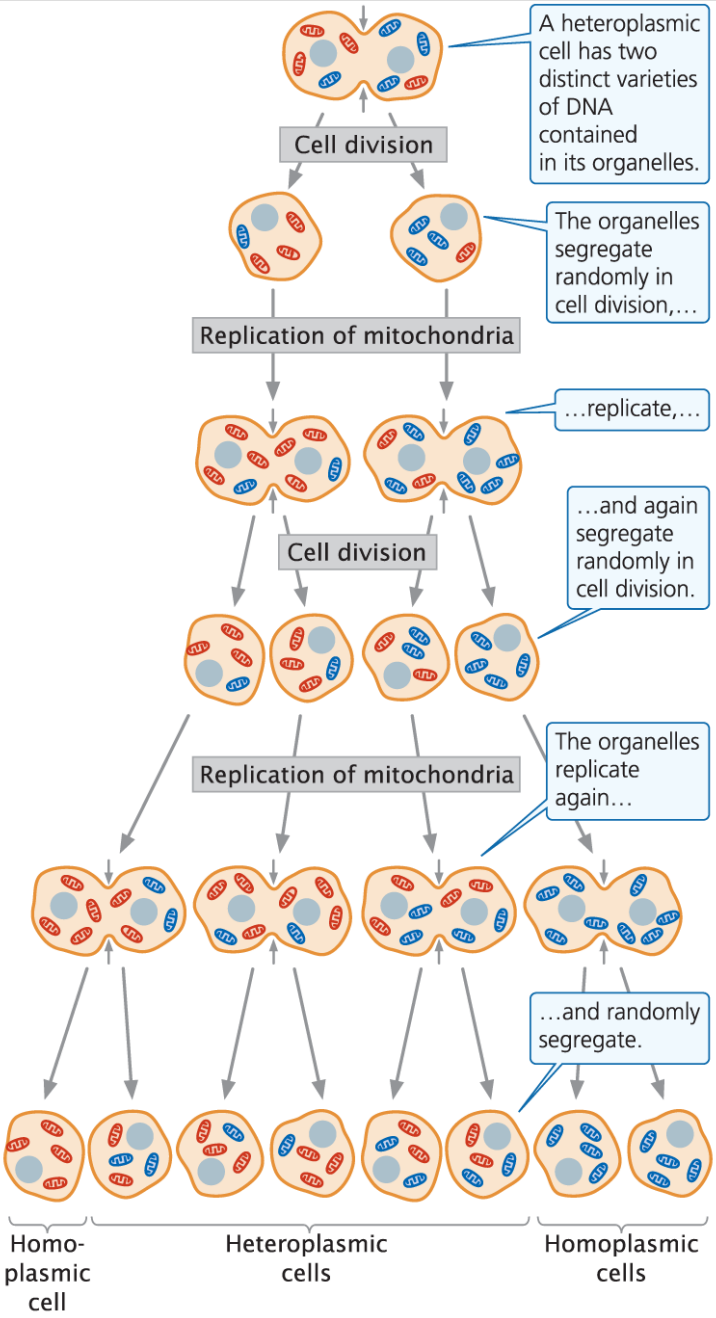
Individual cells may contain from dozens to hundreds of mitochondria and chloroplasts, each with numerous copies of the organelle genome, so each cell typically possesses from hundreds to thousands of copies of mitochondrial and chloroplast genomes (**Figure 11.13**). A mutation arising within one DNA molecule within one organelle generates a mixture of organelles within the cell, some

with a mutant DNA sequence and others with a wild-type DNA sequence. The occurrence of two distinct varieties of DNA within the cytoplasm of a single cell is termed **heteroplasmy**. When a heteroplasmic cell divides, the organelles segregate randomly into the two progeny cells in a process called **replicative segregation** (**Figure 11.14**), and chance determines the proportion of mutant organelles in each cell. Although most progeny cells inherit a mixture of mutant and wild-type organelles, some cells, just by chance, may receive organelles with only mutant or only wild-type sequences; the result, in which all organelles are genetically identical, is known as **homoplasmy**. Fusion of mitochondria also takes place frequently.



Reprinted by permission of The Company of Biologists, from Hayashi, Y. and Ueda, K. 1989. The shape of mitochondria and the number of mitochondrial nucleoids during the cell cycle of *Euglena gracilis, Journal of Cell Science,* 93, p. 565, Figure 3. Permission conveyed through Copyright Clearance Center, Inc.

11.13 Individual cells may contain many mitochondria, each with several copies of the mitochondrial genome. Shown here is a cell of *Euglena gracilis*, a protist, stained so that the nucleus appears red, mitochondria green, and mtDNA yellow. [Reprinted by permission of The Company of Biologists,]



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11.14 Organelles in a heteroplasmic cell segregate randomly into the progeny cells. This diagram illustrates replicative segregation in mitosis; the same process also takes place in meiosis.

Description

The illustration shows a dividing heteroplasmic cell at the top. A caption reads, a heteroplasmic cell has two distinct varieties of D N A contained in its organelles. Cell division occurs and the cell divides into two daughter cells below: one has 3 red mitochondria and 1 blue mitochondria while other has 3 blue and 1 red mitochondria. A caption reads, the organelles segregate randomly in cell division. Replication of mitochondria occurs and each daughter cell starts dividing into two daughters cells. A caption reads, replicate. Cell division occurs. The first cell gives two daughter cells: both with 3 red and 1 blue mitochondria. The second cell gives two daughter cells: one with 4 blue mitochondria and one with 2 blue and 3 red mitochondria. A caption reads, and again segregate randomly in cell division. Replication of mitochondria occurs and each daughter cell starts dividing into two daughters cells as a furrow starts developing. The first cell has two compartments: 4 red mitochondria; 2 blue and 2 red mitochondria.

The second cell has two compartments: 3 red and 1 blue mitochondria; 3 red and 1 blue mitochondria.

The third cell has two compartments: 2 blue and 2 red mitochondria; 2 blue and 2 red mitochondria.

The fourth cell has two compartments: 4 blue mitochondria; 4 blue mitochondria. A caption reads, the organelles replicate again and randomly segregate. The first cell produces two cells below with: 4 red mitochondria (homoplasmic cell); 2 blue and 2 red mitochondria (heteroplasmic cell).

The second cell produces two cells below with: 3 red and 1 blue mitochondria (heteroplasmic cell); 3 red and 1 blue mitochondria (heteroplasmic cell).

The third cell produces two cells below with: 2 blue and 2 red mitochondria (heteroplasmic cell); 2 blue and 2 red mitochondria (heteroplasmic cell).

The fourth cell produces two cells below with: 4 blue mitochondria (homoplasmic cell); 4 blue mitochondria (homoplasmic cell).

A caption below reads, Conclusion: Most of the resulting cells are heteroplasmic, but just by chance, some cells may receive only one type of organelle (e.g., they may receive all normal or all mutant).

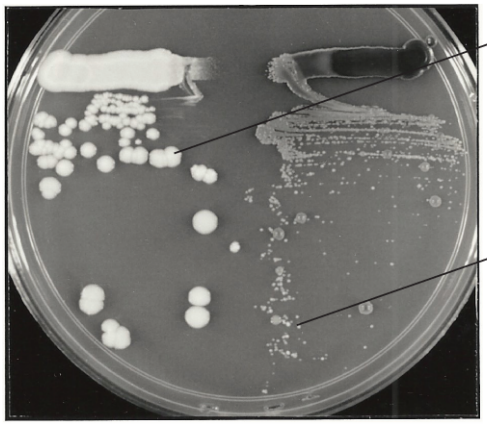
When replicative segregation takes place in somatic cells, it may create phenotypic variation within a single organism: different cells of the organism may possess different proportions of mutant and wild-type sequences, resulting in different degrees of phenotypic expression in different tissues. When replicative segregation takes place in the germ cells of a heteroplasmic cytoplasmic donor, there may be different phenotypes among the offspring.

The disease known as myoclonic epilepsy and ragged-red fiber (MERRF) syndrome is caused by a mutation in an mtDNA gene. In one case, a 20-year-old person who carried this mutation in 85% of his mtDNA sequences displayed a normal phenotype, whereas a cousin who had the mutation in 96% of his mtDNA sequences was severely affected. In diseases caused by mutations in mtDNA, the severity of the disease is frequently related to the proportion of mutant mtDNA sequences inherited at birth. › **TRY** PROBLEM 29

TRAITS ENCODED BY mtDNA

A number of traits affected by organelle DNA have been studied. One of the first to be examined in detail was the phenotype produced by *petite* mutations in yeast (Figure 11.15). In the late 1940s, Boris Ephrussi and his colleagues noticed that when they grew yeast on a solid medium, some colonies were much smaller than normal. Examination of these *petite* colonies revealed that the growth rates of the cells within the colonies were greatly reduced. The results of

biochemical studies demonstrated that the *petite* mutants were unable to carry out aerobic respiration; they obtained all their energy from anaerobic metabolism (glycolysis and fermentation), which is much less efficient than aerobic respiration and results in a smaller colony size.



Reprinted by permission of Genetics Society of America, from Xin Jie Chen and G. Desmond Clark-Walker, The mitochondrial genome integrity gene, *MGI1*, of *Kluyveromyces lactis* encodes the β-subunit of F1-ATPase," *Genetics* 1997, 144 (4): 1445–1454, Figure 1. Courtesy of Xin Jie Chen, Department of Biochemistry and Molecular Biology, SUNY Upstate Medical University. Permission conveyed through Copyright Clearance Center, Inc.

11.15 *Petite* mutations are deletions in mtDNA. Colonies of normal yeast cells and colonies of *petite* mutants are shown here. The *petite* mutants have large deletions in their mtDNA and are unable to carry out oxidative phosphorylation.

Some *petite* mutations are defects in nuclear DNA, but most *petite* mutations occur in mtDNA. Mitochondrial *petite* mutants often have large deletions in mtDNA or, in some cases, are missing mtDNA entirely. Much of the mtDNA sequence encodes enzymes that catalyze aerobic respiration; therefore, the *petite* mutants are unable

to carry out aerobic respiration and cannot produce normal quantities of ATP, which inhibits their growth.

Another group of mtDNA mutations occurs in *Neurospora* (see Chapter 15). Isolated by Mary Mitchell in 1952, *poky* mutants grow slowly, display cytoplasmic inheritance, and have abnormal amounts of cytochromes. Cytochromes are protein components of the electron-transport chain of the mitochondria and play an integral role in the production of ATP. Most organisms have three primary types of cytochromes: cytochrome *a*, cytochrome *b*, and cytochrome *c*. *Poky* mutants have cytochrome *c*, but no cytochrome *a* or *b*. Like *petite* mutants, *poky* mutants are defective in ATP synthesis and therefore grow more slowly than do normal, wild-type cells. **› TRY PROBLEM 33**

A number of genetic diseases that result from mutations in mtDNA (in addition to MERRF syndrome, mentioned earlier) have been identified in humans. Leber hereditary optic neuropathy (LHON) results from mutations in the mtDNA genes that encode electron-transport proteins. LHON typically leads to sudden loss of vision in middle age. Another disease caused by mtDNA mutations is neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP), which is characterized by seizures, dementia, and developmental delay. Other mitochondrial diseases include Kearns–Sayre syndrome (KSS) and chronic external ophthalmoplegia (CEOP), both of which result in paralysis of the eye muscles; droopy eyelids; and, in severe cases, vision loss, deafness, and dementia. All of these

diseases exhibit cytoplasmic inheritance and variable expression (see Chapter 5).

A trait in plants that is produced by mutations in mitochondrial genes is cytoplasmic male sterility, a mutant phenotype found in more than 140 different plant species and inherited only from the maternal parent. These mutations inhibit pollen development but do not affect female fertility.

A number of cpDNA mutants have also been discovered. One of the first to be recognized was the mutation responsible for leaf variegation in the four o'clock plant, *Mirabilis jalapa*, which was studied by Carl Correns in 1909 (see pp. 131–132 in Chapter 5). In the green alga *Chlamydomonas*, streptomycin-resistant mutations occur in cpDNA, and in higher plants, a number of mutants exhibiting altered pigmentation and growth have been traced to defects in cpDNA.

CONCEPTS

In most organisms, genes encoded by mtDNA and cpDNA are inherited from a single parent. A cell may contain more than one distinct type of mtDNA or cpDNA; in these cases, replicative segregation of the organelle DNA may produce phenotypic variation within a single organism, or it may produce different degrees of phenotypic expression among progeny.

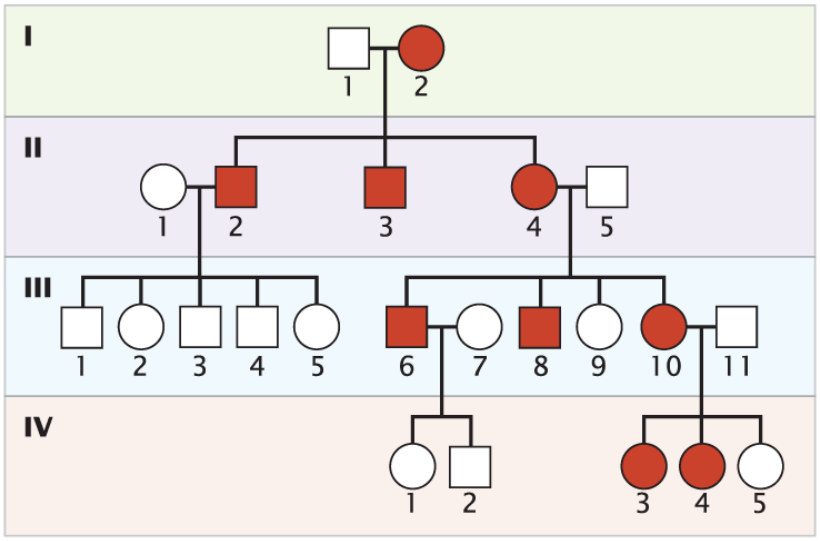
✔ CONCEPT CHECK 9

In a few organisms, traits encoded by mtDNA can be inherited from either parent. This observation suggests that in these organisms,

a. mitochondria do not exhibit replicative segregation.
b. heteroplasmy is present.
c. both sperm and eggs contribute mitochondria to the zygote.
d. there are multiple copies of mtDNA in each cell.

WORKED PROBLEM

A physician examines a young man who has a progressive muscle disorder and visual abnormalities. A number of the patient's relatives have the same condition, as shown in the following pedigree. The degree of expression of the trait is highly variable among members of the family: some are only slightly affected, whereas others developed severe symptoms at an early age. The physician concludes that this disorder is due to a mutation in the mitochondrial genome. Do you agree with the physician's conclusion? Why or why not? Could the disorder be due to a mutation in a nuclear gene? Explain your reasoning.



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Description
In generation 1, the female parent is affected and the male parent is unaffected. They mate and have 2 affected sons and 1 affected daughter in generation 2. One affected male from generation 2 mates with an unaffected female, and have 3 unaffected sons and 2 unaffected daughters in generation 3. The affected female from generation 2 mates with an unaffected male and has 2 affected sons, 1 unaffected daughter, and 1 affected daughter in generation 3. One affected male from generation 3 mates with an unaffected female and has 1 unaffected daughter and 1 unaffected son in generation 4. The affected female from generation 3 mates with an unaffected male and has 2 affected daughters and 1 unaffected daughter in generation 4.

Solution Strategy

What information is required in your answer to the problem?

An explanation of whether and how a mutation in the mitochondrial genome could have caused the patient's disorder, as well as an

explanation of whether and how the disorder could have been caused by a mutation in a nuclear gene.

What information is provided to solve the problem?

- The patient has a progressive muscle disorder and visual abnormalities.
- A pedigree for the patient's family.
- The trait exhibits variable expression among members of the family.

Solution Steps

The conclusion that the disorder is caused by a mutation in the mitochondrial genome is supported by the pedigree and by the observation of variable expression in affected members of the same family. The disorder is passed only from affected mothers to both their male and their female offspring; when fathers are affected, none of their children have the trait (as seen in the children of II-2 and III-6). This outcome is expected of traits determined by mutations in mtDNA because mitochondria are located in the cytoplasm and are usually inherited from a single parent (in humans, the mother). The trait cannot be X-linked recessive because in that case, a cross between a female with the trait (X^aX^a) and a male without the trait (X + Y) would *not* produce daughters with the trait (X^aX^a) which we see in II-4, III-10, IV-3, and IV-4. It cannot be X-linked dominant, because in that case, II-2 and III-6 would have

passed it to their daughters, who are unaffected (unless the trait exhibited incomplete penetrance).

The facts that some offspring of affected mothers do not show the trait (III-9 and IV-5) and that expression varies from one person to another suggest that the affected family members are heteroplasmic, with both mutant and wild-type mitochondria. Replicative segregation of mitochondria in meiosis may produce gametes having different proportions of mutant and wild-type mtDNA sequences, resulting in different degrees of phenotypic expression of the disorder among the offspring. Most likely, symptoms of the disorder develop when some minimum proportion of the mitochondria are mutant. Just by chance, some of the gametes produced by an affected mother contain few mutant mitochondria and result in offspring who lack the disorder.

Another possible explanation for the disorder is that it results from an autosomal dominant allele. When an affected (heterozygous) person mates with an unaffected (homozygous) person, about half of the offspring are expected to have the trait, but just by chance, some affected parents will have no affected offspring. Affected individuals II-2 and III-6 in the pedigree could have just happened to be male, and their sex could be unrelated to the mode of transmission. The variable expression could be explained by variable expressivity (see Chapter 5).

» For more experience with the inheritance of organelle-encoded traits, try working **Problem 30** at the end of the chapter.

The Mitochondrial Genome

In most animals and fungi, the entire mitochondrial genome exists on a single double-stranded, highly coiled, circular DNA molecule, although there may be many copies of this genome in each cell. This circular mitochondrial DNA molecule is similar in structure to a bacterial chromosome. Plant mitochondrial genomes often exist as a complex collection of multiple circular DNA molecules. In some species, the mitochondrial genome consists of a single linear DNA molecule.

Each mitochondrion contains multiple copies of the mitochondrial genome, and a cell may contain many mitochondria. A typical rat liver cell, for example, has from 5 to 10 mtDNA molecules in each of about 1000 mitochondria, so each cell possesses from 5000 to 10,000 copies of the mitochondrial genome. Mitochondrial DNA constitutes about 1% of the total cellular DNA in a rat liver cell. Like bacterial chromosomes, mtDNA lacks the histone proteins normally associated with eukaryotic nuclear DNA, although it is complexed with other proteins that have some histone-like properties. The guanine–cytosine (G–C) content of mtDNA is often sufficiently

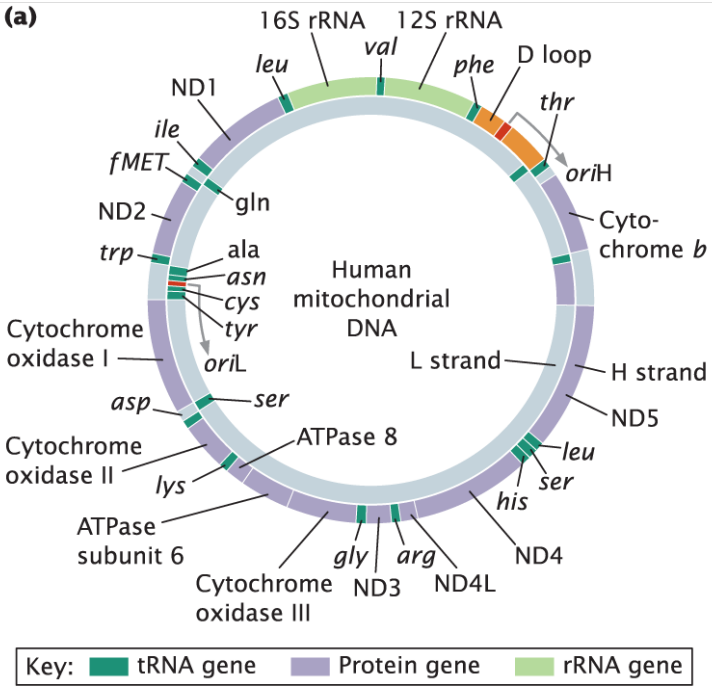
different from that of nuclear DNA that mtDNA can be separated from nuclear DNA by density-gradient centrifugation.

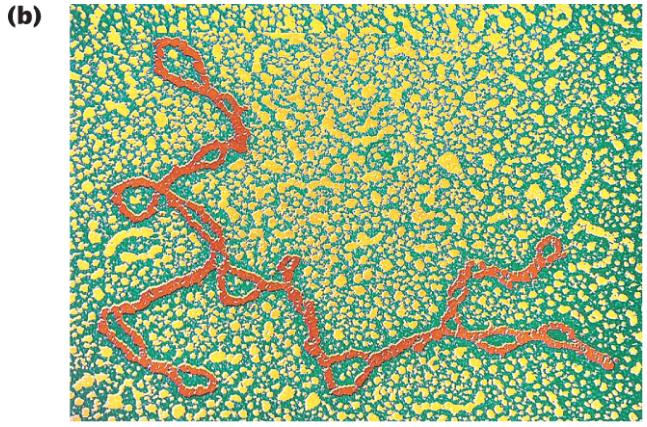
Mitochondrial genomes are small compared with nuclear genomes and vary greatly in size among different organisms. The sizes of mitochondrial genomes of most species range from 15,000 bp to 65,000 bp, but those of a few species are much smaller (e.g., the genome of *Plasmodium falciparum*, the parasite that causes malaria, is only 6000 bp), while those of some plants are several million base pairs. There is no correlation, however, between genome size and number of genes. The number of genes is more constant than genome size: most species have only 40 to 50 genes. These genes encode five basic functions: respiration and oxidative phosphorylation, translation, transcription, RNA processing, and the import of proteins into mitochondria. Most of the variation in the size of mitochondrial genomes is due to differences in noncoding DNA sequences. As mentioned earlier, genes for most of the proteins and enzymes found in mitochondria are actually encoded by nuclear DNA.

HUMAN mtDNA

Human mtDNA is a circular molecule encompassing 16,569 bp that encode 2 rRNAs, 22 tRNAs, and 13 proteins. The two nucleotide strands of the molecule differ in their base composition: the heavy (H) strand has more guanine nucleotides, whereas the light (L)

strand has more cytosine nucleotides. The H strand is the template for both rRNAs, 14 of the 22 tRNAs, and 12 of the 13 proteins, whereas the L strand serves as the template for only 8 of the tRNAs and 1 protein. The **D loop** (**Figure 11.16**) is a region of the mtDNA that contains sites where replication and transcription of the mtDNA is initiated. Human mtDNA is highly economical in its organization: there are few noncoding nucleotides between the genes, and almost all the mRNA transcribed from it encodes proteins. Human mtDNA also contains very little repetitive DNA. The one region of human mtDNA that does contain some noncoding nucleotides is the D loop.





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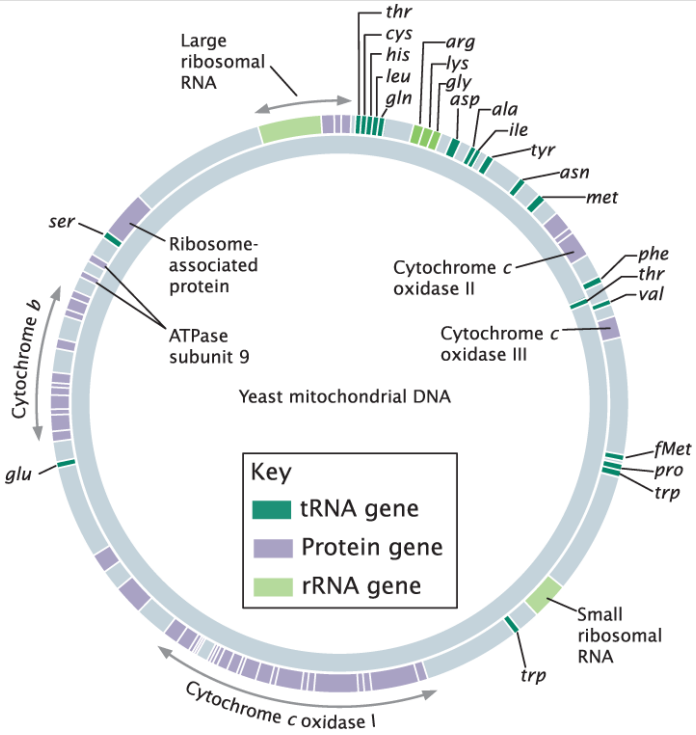
11.16 The human mitochondrial genome, consisting of 16,569 bp, is highly economical in its organization, with few sequences that do not code for RNA or protein. (a) The outer circle represents the heavy (H) strand, and the inner circle represents the light (L) strand. The origins of replication for the H and L strands are *ori*H and *ori*L, respectively. ND identifies genes that encode subunits of NADH dehydrogenase. (b) Electron micrograph of isolated mtDNA.

Description

The diagram “a” shows the protein encoding regions that are colored in purple and the r R N A and t R N A genes are colored in different shades of green. Almost the entire D N A encodes for a gene. The outside of the circle is known as heavy (H) strand and has the majority of the genes, and the light (L) strand encodes very few genes. The origins for the heavy and light strands are on opposite sides of the plasmid. Genes N D 1, 2, 3, 4, 4 L, and 5 are labeled in the diagram. The electron micrograph below labeled “b”, shows isolated mitochondrial D N A (m t D N A).

YEAST mtDNA

The organization of yeast (*Saccharomyces cerevisiae*) mtDNA is quite different from that of human mtDNA. Although the yeast mitochondrial genome, with its 78,000 bp, is nearly five times as large, it encodes only six additional genes, for a total of 2 rRNAs, 25 tRNAs, and 16 proteins (**Figure 11.17**). Most of the extra DNA in the yeast mitochondrial genome consists of noncoding sequences found within and between genes.



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11.17 The yeast mitochondrial genome, consisting of 78,000 bp, contains much noncoding DNA.

Description
The D N A shows regions for the protein gene, r R N A gene, and t R N A gene. The outer strand shows most of the coding regions, such as: a s n, m e t, v a l, t r p. The inner strand has most of the noncoding regions.

FLOWERING-PLANT mtDNA

Flowering plants (angiosperms) have the largest and most complex mitochondrial genomes known: their mitochondrial genomes range in size from 186,000 bp in white mustard to 2,400,000 bp in

muskmelon. Even closely related plant species may differ greatly in the sizes of their mtDNA. Part of the extensive size variation in the mtDNA of flowering plants can be explained by the presence of long sequences that are direct repeats. Crossing over between these repeats can generate multiple circular chromosomes of different sizes.

CONCEPTS

The mitochondrial genome consists of circular DNA with no associated histone proteins, although it is complexed with other proteins that have some histone-like properties. The sizes and structures of mtDNA differ greatly among organisms. Human mtDNA exhibits extreme economy, but mtDNAs found in yeast and flowering plants contain many noncoding nucleotides and repetitive sequences. In most flowering plants, mitochondrial DNA is large and typically has one or more large direct repeats that can recombine to generate smaller or larger molecules.

The Evolution of Mitochondrial DNA

As already mentioned, comparisons of mitochondrial DNA sequences with DNA sequences in bacteria strongly support a common bacterial origin for all mtDNA. Nevertheless, patterns of evolution seen in mtDNA vary greatly among different groups of organisms.

The sequences of vertebrate mtDNA exhibit an accelerated rate of evolution: the sequences in mammalian mtDNA, for example, typically change from 5 to 10 times faster than those in mammalian nuclear DNA. The accelerated rate of evolution seen in vertebrate mtDNA is due to its high mutation rate, which allows sequences to change more quickly. In spite of this high rate of sequence evolution, the numbers of genes present and the organization of vertebrate mitochondrial genomes are relatively constant. In contrast, the sequences of plant mtDNA evolve slowly, at a rate only one-tenth that of the nuclear genome, but their gene content and organization change rapidly. The reason for these basic differences in rates of evolution is not yet known.

Mitochondrial DNA has been studied extensively to reconstruct patterns of evolution in humans and many other organisms. Some of the advantages of mtDNA for studying evolution include (1) the small size of mtDNA and its abundance in the cell; (2) the rapid evolution of mtDNA sequences in some organisms, which facilitates study of closely related groups; and (3) the maternal inheritance of mtDNA and lack of recombination, which makes it possible to trace female lines of descent. Samples of human mtDNA from thousands of people belonging to hundreds of different ethnic groups throughout the world have been analyzed. These mtDNA samples are helping unravel many aspects of human evolution and history. For example, initial studies on mtDNA sequences led to the proposal that small groups of humans migrated out of Africa about 85,000 years ago and populated the rest of the world. This proposal, called

the Out of Africa hypothesis or the African replacement hypothesis, has now gained wide acceptance. The Out of Africa hypothesis is supported by additional studies of DNA sequences from the Y chromosome and nuclear genes. The use of mtDNA in evolutionary studies will be described in more detail in Chapter 26.

At conception, a mammalian zygote inherits approximately 100,000 copies of mtDNA from the egg. Because of the large number of mtDNA molecules in each cell and the high rate of mutation in mtDNA, most cells would be expected to contain a mixture of wild-type and mutant mtDNA molecules (heteroplasmy). However, heteroplasmy is rarely present: the copies of mtDNA in most individuals are genetically identical (homoplasmy). To account for the uniformity of mtDNA within individual mammals, geneticists hypothesize that at some point in early development or gamete formation, mtDNA goes through some type of bottleneck, during which the mtDNAs within a cell are reduced to just a few copies, which then replicate and give rise to all subsequent copies of mtDNA. Through this process, genetic variation in mtDNA within a cell is eliminated.

THINK-PAIR-SHARE Question 3

CONCEPTS

All mtDNA appears to have evolved from a common bacterial ancestor, but the patterns of evolution seen in different mitochondrial genomes vary greatly. Vertebrate mtDNA exhibits

rapid change in sequence but little change in gene content and organization, whereas the mtDNA of plants exhibits little change in sequence but much variation in gene content and organization. Mitochondrial DNA sequences are frequently used to study patterns of evolution.

Damage to Mitochondrial DNA Associated with Aging

The symptoms of many human genetic diseases caused by defects in mtDNA first appear in middle age or later and increase in severity as people age. One hypothesis to explain this pattern is related to the decline in oxidative phosphorylation with aging.

Oxidative phosphorylation is the process that generates ATP, the primary carrier of energy in the cell. This process takes place on the inner membrane of the mitochondrion and requires a number of different proteins, some encoded by mtDNA and others encoded by nuclear genes. Oxidative phosphorylation normally declines with age, and if it falls below a critical threshold, tissues do not make enough ATP to sustain vital functions and disease symptoms appear. Most people start life with an excess capacity for oxidative phosphorylation; this capacity decreases with age, but most people reach old age or die before the critical threshold is passed. People who are born with mitochondrial diseases carry mutations in their mtDNA that lower their capacity for oxidative phosphorylation. At birth, their capacity may be sufficient to support their ATP needs,

but as their oxidative phosphorylation capacity declines with age, they cross the critical threshold and begin to experience disease symptoms.

The mechanism of age-related increases in mtDNA damage is not yet known. Oxygen radicals—highly reactive compounds that are natural by-products of oxidative phosphorylation—are known to damage DNA (see p. 541 in Chapter 18). Because mtDNA is physically close to the enzymes taking part in oxidative phosphorylation, mtDNA may be more prone to oxidative damage than is nuclear DNA. When mtDNA has been damaged, the cell's capacity to produce ATP drops.

Mitochondrial Replacement Therapy

A number of severe genetic disorders result from mutations in mtDNA, and 1 out of every 5000 babies is born with a mitochondrial disease. Geneticists have developed methods that allow a woman carrying an mtDNA mutation to give birth to a healthy child. Called **mitochondrial replacement therapy (MRT)**, these methods combine the nuclear DNA of a female who carries a mitochondrial mutation with that of a sperm and the egg cytoplasm of a healthy donor, creating a "three-parent baby."

One type of MRT, termed pronuclear transfer, starts with an egg from a woman with an mtDNA mutation. The egg is fertilized in vitro (in the laboratory). After penetrating the egg, the sperm releases its

nucleus into the egg, resulting in two nuclei (called pronuclei), one from the father and one from the mother. Using a very fine pipette, a technician removes the pronuclei and transfers them to an egg cell from a healthy donor from which the nucleus has been previously removed. Inside the donor egg, the pronuclei fuse, and the resulting embryo begins development. The developing embryo is then implanted into the uterus of the affected female.

In a related technique, called maternal spindle transfer, the nuclear material is removed from the unfertilized oocyte of an affected female and transferred to an enucleated donor egg. The resulting oocyte, containing nuclear genetic material from the affected mother and cytoplasm from the healthy donor, is fertilized in vitro. The resulting embryo is then implanted into the affected female.

Mitochondrial replacement therapy is legally permissible for preventing mitochondrial disorders in the United Kingdom and a few other countries, but its use is not currently allowed in the United States. Because it combines genetic material from three parents in the conception of a child, MRT has raised a number of ethical and safety issues. Some mutated mitochondrial DNAs may be carried over when the nuclear material is transferred to a healthy egg; although this is usually a very small fraction of all the mtDNA in the cell, the long-term consequences are unknown. There have also been concerns about the possible developmental consequences of combining nuclear DNA and mtDNA from different people.

THINK-PAIR-SHARE Question 4

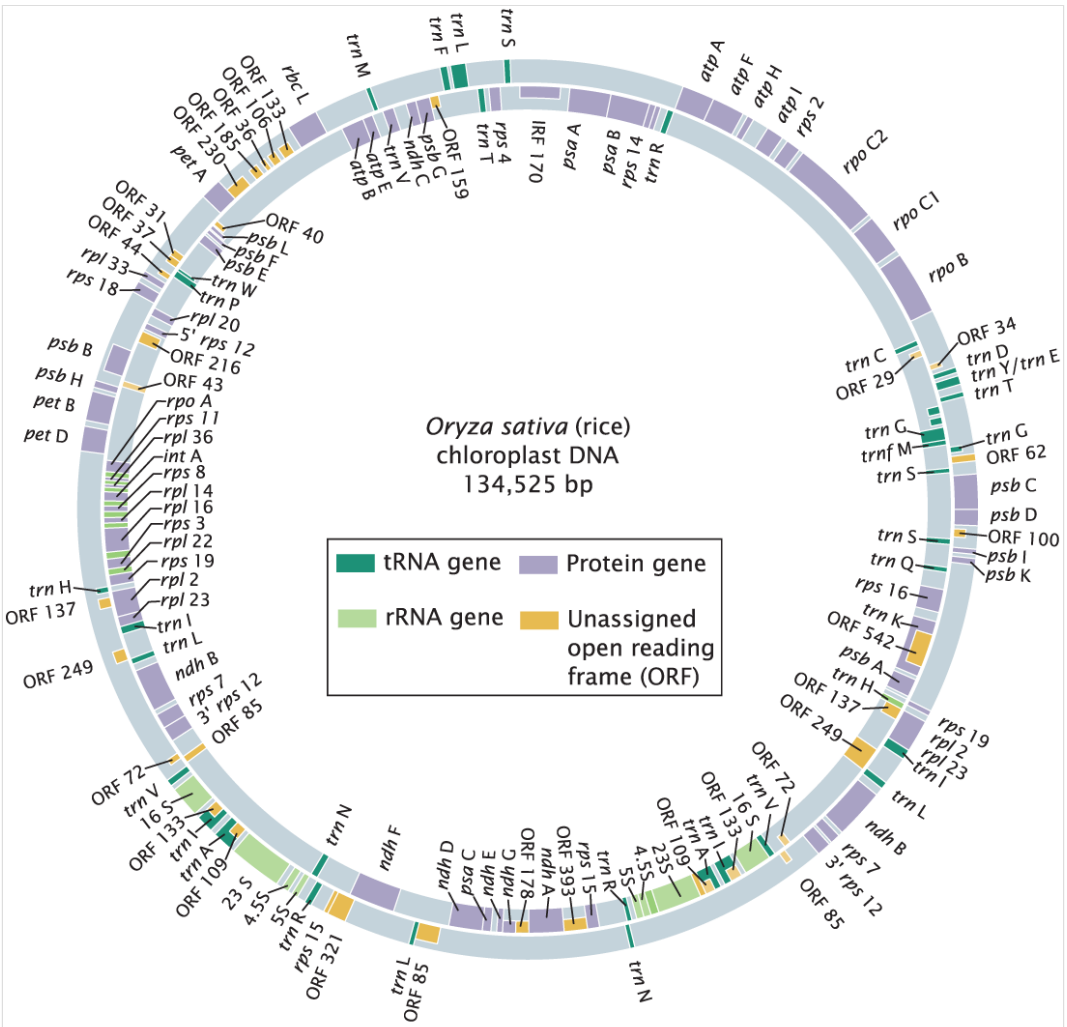
The Chloroplast Genome

Among plants, the chloroplast genome ranges in size from 80,000 to 600,000 bp, but most chloroplast genomes range from 120,000 to 160,000 bp. Chloroplast DNA is usually a single double-stranded DNA molecule that is circular, highly coiled, and lacks associated histone proteins. As in mtDNA, multiple copies of the chloroplast genome are found in each chloroplast, and there are multiple organelles per cell, so there are several hundred to several thousand copies of cpDNA in a typical plant cell.

The chloroplast genomes of a number of plant and algal species have been sequenced, and cpDNA is now recognized to be basically bacterial in its organization: the order of some groups of genes is the same as that observed in *E. coli*, and many chloroplast genes are organized into clusters similar to those found in bacteria. Many of the gene sequences in cpDNA are quite similar to those found in homologous bacterial genes.

Among vascular plants, chloroplast genomes are similar in gene content and gene order. A typical chloroplast genome encodes 4 rRNA genes, from 30 to 35 tRNA genes, a number of ribosomal proteins, many proteins engaged in photosynthesis, and several

proteins with roles in nonphotosynthetic processes (Figure 11.18). A key protein encoded by cpDNA is ribulose-1,5-bisphosphate carboxylase-oxygenase (abbreviated RuBisCO), which participates in the fixation of carbon in photosynthesis. RuBisCO makes up about 50% of the protein found in green plants and is therefore considered the most abundant protein on Earth. It is a complex protein consisting of eight identical large subunits and eight identical small subunits. The large subunit is encoded by chloroplast DNA, whereas the small subunit is encoded by nuclear DNA. Much of cpDNA consists of noncoding sequences.



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11.18 Chloroplast DNA of rice. An unassigned open reading frame (ORF) refers to an apparent protein-encoding gene—a sequence with a translation start and stop signal in the same reading frame—for which the protein has not yet been identified.

Description

The D N A shows regions for protein genes, t R N A genes, r R N A genes, and unassigned open reading frame (O R F's). There are a large number of genes labeled on the outer strand while remaining regions are non-coding. Some of the outer strand genes are: a t p A, r p o C 1, t r n N, and p e t B. A large number of genes are also labeled on the inner strand while remaining regions are noncoding. Some of the inner strand genes are: t r n C, n d h F, i n t A, and r p o A.

THE EVOLUTION OF CHLOROPLAST DNA

The DNA sequences of chloroplasts are very similar to those found in cyanobacteria (a group of photosynthetic bacteria), so chloroplast genomes clearly have a bacterial ancestry. Overall, cpDNA sequences evolve slowly compared with sequences in nuclear DNA and some mtDNA. For most chloroplast genomes, size and gene organization are similar, although there are some notable exceptions. Because it evolves slowly and, like mtDNA, is inherited from only one parent, cpDNA is often useful for determining the evolutionary relationships among different plant species.

CONCEPTS

Most chloroplast genomes consist of a single circular DNA molecule not complexed with histone proteins. Although there is considerable size variation among species, the chloroplast genomes found in most plants range from 120,000 to 160,000 bp. Chloroplast DNA sequences are most similar to DNA sequences in cyanobacteria, which supports the endosymbiotic theory of chloroplast origin.

✔ CONCEPT CHECK 10

In its organization, chloroplast DNA is most similar to

a. bacteria.
b. archaea.
c. nuclear DNA of plants.
d. nuclear DNA of primitive eukaryotes.

Movement of Genetic Information Between Nuclear, Mitochondrial, and Chloroplast Genomes

As we have seen, many of the proteins found in modern mitochondria and chloroplasts are encoded by nuclear genes, which suggests that much of the genetic material in the original endosymbionts has been transferred to the nucleus over evolutionary time. This assumption is also supported by the observation that some DNA sequences normally found in mtDNA have been detected in the nuclear DNA of some strains of yeast and corn. Likewise, chloroplast sequences have been found in the nuclear DNA of spinach. Furthermore, the sequences of those nuclear genes that encode organelle proteins are most similar to their bacterial counterparts.

There is also evidence that during evolution, genetic material has moved from chloroplasts to mitochondria. For example, DNA fragments from some rRNA genes that are normally found in cpDNA have been found in the mtDNA of corn. Sequences from the gene that encodes the large subunit of RuBisCO, which is normally encoded by cpDNA, are duplicated in corn mtDNA. And there is even evidence that some nuclear genes have moved into mitochondrial genomes. The exchange of genetic material between the nuclear, mitochondrial, and chloroplast genomes over evolutionary time has given rise to the term “promiscuous DNA” to describe this

phenomenon. The mechanism by which this exchange takes place is not entirely clear.

Chapter 11 Review

CHAPTER SUMMARY

Section 11.1 Learning Objective: Be able to explain how large amounts of DNA are packed into a cell.

- Chromosomes contain very long DNA molecules that are tightly packed.
- Supercoiling results from strain produced when rotations are added to a relaxed DNA molecule or removed from it. Overrotation produces positive supercoiling; underrotation produces negative supercoiling. Supercoiling is controlled by topoisomerase enzymes.
- A bacterial chromosome consists of a single circular DNA molecule that is bound to proteins and exists as a series of large loops. It usually appears in the cell as a distinct clump known as the nucleoid.
- Each eukaryotic chromosome contains a single long linear DNA molecule that is bound to histone and nonhistone chromosomal proteins. Euchromatin undergoes the normal cycle of decondensation and condensation in the cell cycle. Heterochromatin remains highly condensed throughout the cell cycle.

- The nucleosome is a core particle of eight histone proteins and the DNA that wraps around them. Nucleosomes are folded into a 30-nm fiber that forms a series of 300-nm-long loops; these loops are anchored at their bases by proteins. The 300-nm loops are condensed to form a fiber that is itself tightly coiled to produce a chromatid.
- Topologically associated domain (TADs) are areas of chromatin that form regions of spatial association within the interphase nucleus.
- Chromosome regions that are undergoing active transcription are sensitive to digestion by DNase I, indicating that DNA is more exposed during transcription.
- Epigenetic changes are stable alterations of gene expression that do not require changes in DNA sequences. Epigenetic changes can take place through alterations of chromatin structure.

Section 11.2 Learning Objective: Be able to describe the special features of centromeres and telomeres.

- Centromeres are chromosomal regions where spindle microtubules attach; chromosomes without centromeres are usually lost in the course of cell division. Most centromeres are defined by epigenetic changes to chromatin structure. Telomeres stabilize the ends of chromosomes.

Section 11.3 Learning Objective: Be able to describe different types of sequences found in eukaryotic chromosomes.

- Eukaryotic DNA comprises three classes of sequences. Unique-sequence DNA exists in very few copies. Moderately repetitive DNA consists of moderately long sequences that are repeated from hundreds to thousands of times. Highly repetitive DNA consists of very short sequences that are repeated in tandem from many thousands to millions of times.

Section 11.4 Learning Objective: Be able to explain how DNA in mitochondria and chloroplasts differs from nuclear DNA.

- Mitochondria and chloroplasts are eukaryotic organelles that possess their own DNA. The endosymbiotic theory proposes that mitochondria and chloroplasts originated as free-living bacteria that entered into a beneficial association with eukaryotic cells.
- Traits encoded by mtDNA and cpDNA are usually inherited from a single parent, most often the mother. Replicative segregation of organelles in cell division may produce phenotypic variation among cells within an individual organism and among the offspring of a single female.
- The mitochondrial genome usually consists of a single circular DNA molecule that lacks histone proteins. Mitochondrial DNA varies in size among different groups of organisms. Human mtDNA is highly economical, with few noncoding nucleotides. Fungal and plant mtDNAs contain much noncoding DNA between genes.
- Comparisons of mtDNA sequences suggest that mitochondria evolved from a bacterial ancestor. Vertebrate mtDNA exhibits

rapid change in sequence but little change in gene content and organization. Plant mtDNA exhibits little change in sequence but much variation in gene content and organization.

- Mitochondrial DNA sequences are widely used to study evolution. Damage to mtDNA has been associated with aging in humans.
- Mitochondrial replacement therapy can be used to transfer nuclear DNA from a woman with a mitochondrial disorder and from a sperm into an egg cell from a healthy donor, giving rise to a baby with genetic material from three parents. This procedure has raised ethical issues and safety concerns.
- Chloroplast genomes consist of a single circular DNA molecule that lacks histone proteins and varies little in size. Each plant cell contains multiple copies of cpDNA. Chloroplast DNA sequences are most similar to those in cyanobacteria and tend to evolve slowly.
- Through evolutionary time, many mitochondrial and chloroplast genes have moved to nuclear chromosomes. In some plants, there is evidence that copies of chloroplast genes have moved to the mitochondrial genome.

WORKED PROBLEMS

Problem 1

A diploid plant cell contains 2 billion base pairs of DNA.

a. How many nucleosomes are present in the cell?
b. Give the number of molecules of each type of histone protein associated with the genomic DNA.

» Solution Strategy

What information is required in your answer to the problem?

The number of nucleosomes per cell and the number of molecules of each type of histone protein associated with the DNA.

What information is provided to solve the problem?

The cell contains 2 billion base pairs of DNA.

For help with this problem, review:

The Nucleosome in Section 11.1.

» Solution Steps

Each nucleosome encompasses about 200 bp of DNA: 145–147 bp of DNA wrapped around the histone core, 20–22 bp of DNA associated with the H1 protein, and another 30–40 bp of linker DNA.

> **Recall:** The repeating unit of the chromosome is a nucleosome, which consists of DNA complexed with histone proteins.

a. To determine how many nucleosomes are present in the cell, we simply divide the total number of base pairs of DNA (2×10^9 bp) by the number of base pairs per nucleosome:

$$\frac{2\times10^9 \text{ base pairs}}{2\times10^2 \text{ base pairs/nucleosome}} = 1 \times 10^7 \text{ nucleosomes}$$

Thus, there are approximately 10 million nucleosomes in the cell.

b. Each nucleosome includes two molecules each of H2A, H2B, H3, and H4 histones. Therefore, there are 2×10^7 molecules each of H2A, H2B, H3, and H4 histones. Each nucleosome has one copy of the H1 histone associated with it, so there are 1×10^7 molecules of H1.

Problem 2

Suppose that a new organelle is discovered in an obscure group of protists. This organelle contains a small DNA genome, and some scientists are arguing that like chloroplasts and mitochondria, this organelle originated as a free-living eubacterium that entered into an endosymbiotic relation with the protist. Outline a research plan to determine whether the new organelle evolved from a free-living eubacterium. What kinds of data would you collect, and what predictions would you make if the theory were correct?

» Solution Strategy

What information is required in your answer to the problem?

A research plan with the types of data you would collect as well as predictions.

What information is provided to solve the problem?

- The newly discovered organelle contains a small DNA genome.
- Other organelles have probably evolved through an endosymbiotic relationship.

For help with this problem, review:

The Endosymbiotic Theory, The Mitochondrial Genome, and The Chloroplast Genome in Section 11.4.

» Solution Steps

We should examine the structure, organization, and sequences of the organelle genome. If the organelle shows only characteristics of eukaryotic DNA, then it most likely has a eukaryotic origin, but if it displays some characteristics of bacterial DNA, those observations support the theory of a bacterial origin.

> **Recall:** The endosymbiotic theory proposes that organelles evolved from bacteria.

We could start by examining the overall characteristics of the organelle DNA. If it has a bacterial origin, we might expect that the organelle genome will consist of a circular molecule and will lack

histone proteins. We could compare the DNA sequences found in the organelle genome with homologous sequences from bacterial and eukaryotic genomes. If the theory of an endosymbiotic origin is correct, then the organelle sequences should be most similar to homologous sequences found in bacteria.

IMPORTANT TERMS

mitochondrial DNA (mtDNA)
chloroplast DNA (cpDNA)
supercoiling
relaxed state
positive supercoiling
negative supercoiling
topoisomerase
nucleoid
euchromatin
heterochromatin
nonhistone chromosomal protein
nucleosome
linker DNA
topologically associated domains (TADs)
polytene chromosome
chromosome puff
epigenetic change
telomeric sequence
shelterin

C-value
C-value paradox
denaturation
melting temperature
renaturation
hybridization
unique-sequence DNA
gene family
repetitive DNA
moderately repetitive DNA
tandem repeat
interspersed repeat
short interspersed element (SINE)
long interspersed element (LINE)
highly repetitive DNA
endosymbiotic theory
heteroplasmy
replicative segregation
homoplasmy
D loop
mitochondrial replacement therapy

ANSWERS TO CONCEPT CHECKS

1. b
2. Bacterial DNA is not complexed with histone proteins and is circular.

3. b
4. d
5. A chromosome that loses its centromere will not segregate into the nucleus in mitosis and is usually lost.
6. d
7. a
8. Many modern protists are hosts to endosymbiotic bacteria. Mitochondria and chloroplasts are similar in size to bacteria and have their own DNA, as well as ribosomes that are similar in size and shape to bacterial ribosomes. Antibiotics that inhibit protein synthesis in bacteria also inhibit protein synthesis in mitochondria and chloroplasts. Gene sequences in mtDNA and cpDNA are most similar to bacterial DNA sequences.
9. c
10. a

Chapter 11 Assessment

COMPREHENSION QUESTIONS

Section 11.1

1. How does supercoiling arise? What is the difference between positive and negative supercoiling?

2. What functions does supercoiling serve for the cell?

3. Describe the composition and structure of the nucleosome.

4. Describe in steps how the double helix of DNA, which is 2 nm in width, gives rise to a chromosome that is 700 nm in width.

5. What are polytene chromosomes and chromosome puffs?

6. What are epigenetic changes, and how are they brought about?

Section 11.2

7. Describe the function of the centromere. How are centromeres different from other regions of the chromosome?

8. Describe the function and molecular structure of a telomere.

9. What is the difference between euchromatin and heterochromatin?

Section 11.3

10. What is the C-value of an organism?

11. Describe the different classes of DNA sequence variation that exist in eukaryotes.

Section 11.4

12. Explain why many traits encoded by mtDNA and cpDNA exhibit considerable variation in their expression, even among members of the same family.

13. What is the endosymbiotic theory? How does it help to explain some of the characteristics of mitochondria and chloroplasts?

14. What evidence supports the endosymbiotic theory?

15. Briefly describe the organization of genes on the chloroplast genome.

16. What is meant by the term *promiscuous* DNA?

APPLICATION QUESTIONS AND PROBLEMS

Introduction

17. The introduction to this chapter discussed a study of telomere length in Romanian children. The study demonstrated that children raised in orphanages had shorter telomeres than children raised in foster homes. What effect, if any, do you think having shorter telomeres in childhood might have on adult life?

Section 11.1

18. *Compare and contrast bacterial and eukaryotic chromosomes. How are they alike, and how do they differ?

19. *In a typical eukaryotic cell, would you expect to find more molecules of the H1 histone or more molecules of the H2A histone? Would you expect to find more molecules of H2A or more molecules of H3? Explain your reasoning.

20. CONNECT Archaea are prokaryotes, lacking a nucleus and possessing a circular DNA similar to bacteria. However, they have histones and nucleosome-like structures similar to those found in eukaryotes. Why are the archaea more similar to eukaryotes than bacteria in how their DNA is packaged? (Hint: See the discussion of bacteria, archaea, and eukaryotes in Section 2.1 in Chapter 2.)

21. *Based on the sensitivity of DNA to DNase I, as illustrated in **Figure 11.7**, which type of chicken hemoglobin (embryonic or

adult) is likely to be produced in the highest quantity in the following tissues and developmental stages?

a. Erythroblasts during the first 24 hours
b. Erythroblasts at day 5
c. Erythroblasts at day 14
d. Brain cells throughout development

22. *A diploid human cell contains approximately 6.4 billion base pairs of DNA.
    a. How many nucleosomes are present in such a cell? (Assume that the linker DNA encompasses 40 bp.)
    b. How many histone proteins are complexed with this DNA?

23. *Would you expect to see more or less acetylation in regions of DNA that are sensitive to digestion by DNase I? Why?

24.

Gunter Korge examined several proteins that are secreted from the salivary glands of *Drosophila melanogaster* during larval development (G. Korge. 1975. *Proceedings of the National Academy of Sciences of the United States of America* 72:4550–4554). One protein, called protein fraction 4, was encoded by a gene found by deletion mapping to be located on the X chromosome at position 3C. Korge observed that about 5 hours before the first synthesis of protein fraction 4, an expanded and puffed-out region formed on the X chromosome at position 3C. This chromosome puff disappeared before the end of the third larval instar stage, when the synthesis of protein fraction 4 ceased. He observed that there was no puff at position 3C in a special strain of flies that lacked secretion of protein fraction 4. Explain these

results. What is the chromosome puff at region 3, and why does its appearance and disappearance roughly coincide with the secretion of protein fraction 4?

25. Suppose a chemist develops a new drug that neutralizes the positive charges on the tails of histone proteins. What would be the most likely effect of this new drug on chromatin structure? Would this drug have any effect on gene expression? Explain your answers.

Section 11.2

26. CONNECT What would be the likely consequences of a large deletion in the gene that encoded CENP-A, so that no functional CENP-A was produced? (Hint: See Chapters 2 and 8).

Section 11.3

27. *Which of the following two molecules of DNA has the lower melting temperature? Why?

AGTTACTAAAGCAATACATC
TCAATGATTTCGTTATGTAG

AGGCGGGTAGGCACCCTTA
TCCGCCCATCCGTGGGAAT

Description

The first nucleotide pair at the top has the following top strand sequence: A G T T A C T A A A G C A A T A C A T C.

The bottom strand below has the following sequence: T C A A T G A T T T C G T T A T G T A G.

The second nucleotide pair at the bottom has the following top strand sequence: A G G C G G G T A G G C A C C C T T A.

The bottom strand below has the following sequence: T C C G C C C A T C C G T G G G A A T.

28. In a DNA-hybridization study, DNA was isolated from wheat, labeled with ^{32}P, and sheared into small fragments (S. K. Dutta et al. 1967. *Genetics* 57:719–727). Hybridizations between these labeled fragments and denatured DNA from different plant species were then compared. The following table gives the percentages of labeled wheat DNA that hybridized to DNA molecules of wheat, corn, radish, and cabbage.

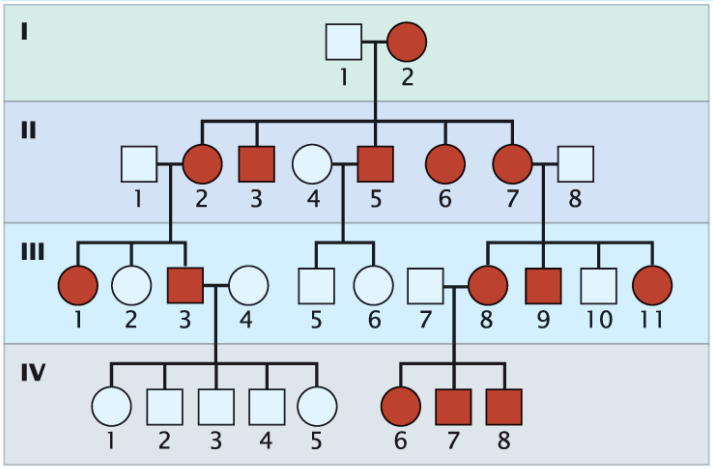
Species	Percentage of wheat DNA hybridized relative to wheat
Wheat	100
Corn	63
Radish	30
Cabbage	23

What do these results indicate about the evolutionary differences among these plants?

Section 11.4

29. *A wheat plant that is light green in color is found growing in a field. Biochemical analysis reveals that chloroplasts in this plant produce only 50% of the chlorophyll normally found in wheat chloroplasts. Propose a set of crosses to determine whether the light-green phenotype is caused by a mutation in a nuclear gene or in a chloroplast gene.

30. CONNECT *The following pedigree illustrates the inheritance of a rare neurological disease (see Chapter 6 for a discussion of pedigrees). What is the most likely mode of inheritance for this disorder? Explain your reasoning.



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Description

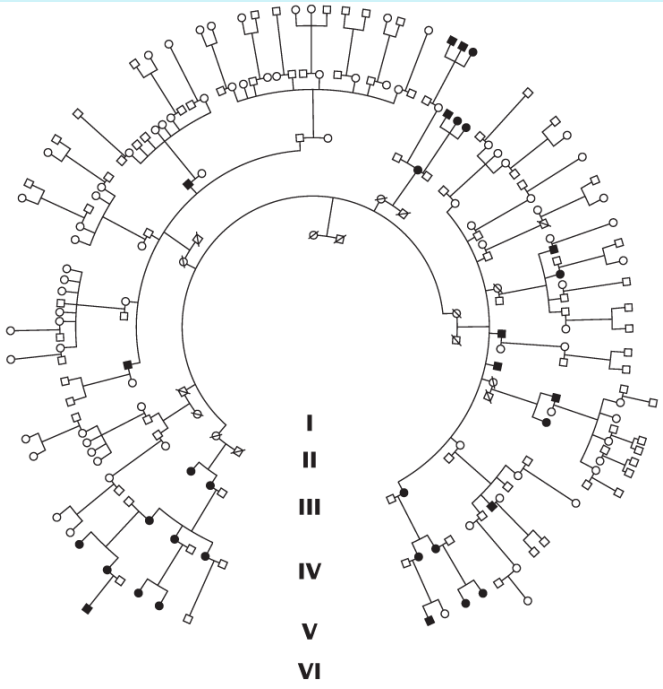
In generation one, the female is affected and the male is unaffected. They mate and have 1 affected daughter and 2 affected sons in generation 2. The affected male from generation 2 mates with an unaffected female and has 3 unaffected sons and 2 unaffected daughters in generation 3. The affected female from generation 2 mates with an unaffected male and has 2 affected sons (one of which is a proband), 1 unaffected daughter, and 1 affected daughter in generation 3. The proband from generation 3 mated with an unaffected daughter and has 1 unaffected son and 1 unaffected daughter in generation 4. The affected female from generation 3 mates with an unaffected male and has 2 affected daughters and 1 unaffected daughter in generation 4.

31. Assume that the muscle disorder whose inheritance is illustrated in the pedigree in the Worked Problem on p. 332 is a rare disease that results from a defect in mitochondrial DNA. If individual III-8 has a daughter, what is the probability that the daughter will inherit the muscle disorder from her affected parent?

32.

Fredrick Wilson and his colleagues studied members of a large family who had low levels of magnesium in their blood (see the pedigree below). They argued that this disorder (and associated high blood pressure and high cholesterol) is caused by a mutation in mtDNA (F. H. Wilson et al. 2004. *Science* 306:1190–1194).
    a. What evidence suggests that a mutation in mtDNA is causing this disorder?
    b. Could this disorder be caused by an autosomal dominant gene? (see Chapter 6 for characteristics of

autosomal dominant traits in pedigrees.) Why or why not?



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[Data from F. H. Wilson et al. 2004. *Science* 306:1190–1194.]

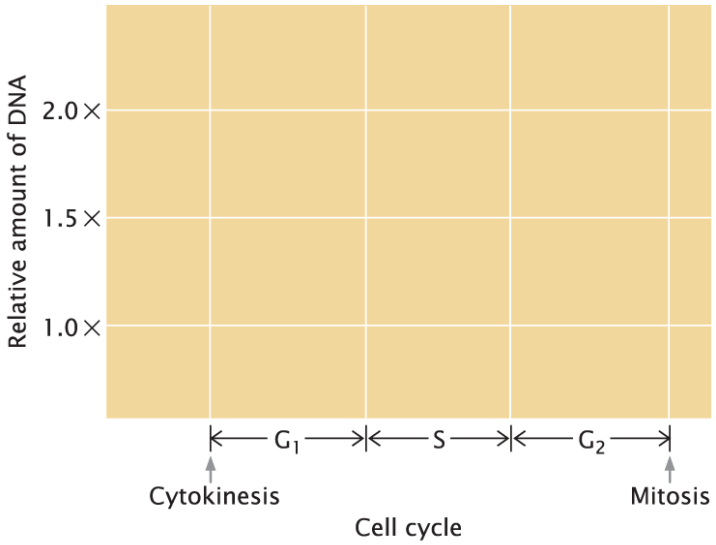
Description

The male crossed out and female crossed out in generation 1 have five female offspring (all crossed out) who all mate with males (all crossed out) and have offspring. None of these individuals are known to be affected with the disorder. Generation 3 has 19 members (9 females and 10 males). Of these 8 are affected (4 males and 4 females). Most of the unaffected members do not pass on the disorder, but 3 of them who are female have offspring that are affected. One female has only unaffected male offspring, one female crossed out has 2 affected offspring, and the other female crossed out has 2 offspring, one affected male and one affected female. No other offspring in these 3 family lineages has the disease. 3 of the 4 males affected with the disease

have children. None of their children are affected. Of the affected females in generation 3, they all mate with unaffected males and have 9 offspring in total, 7 affected females, 1 affected male, and 1 unaffected female. The one affected female mates with an unaffected male and has 3 afflicted offspring. Only 5 of the 7 affected females mate and have offspring. In total, they have 9 offspring: 6 affected females, 1 affected male, 1 unaffected male, and 1 unaffected female. Only one of the females from this generation mates and has children, and they have only one affected male offspring.

33. *In a particular strain of *Neurospora*, a *poky* mutation exhibits biparental inheritance, whereas *poky* mutations in other strains are inherited only from the maternal parent. Explain these results.

34. CONNECT *A scientist collects cells at various points in the cell cycle (see Chapter 2) and isolates DNA from them. Using density-gradient centrifugation, she separates the nuclear DNA and mtDNA. She then measures the amounts of mtDNA and nuclear DNA present at different points in the cell cycle. On the following graph, draw a line to represent the relative amounts of nuclear DNA that you expect her to find per cell throughout the cell cycle. Then draw a dotted line on the same graph to indicate the relative amounts of mtDNA that you would expect to see at different points throughout the cell cycle.



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Description

The horizontal axis is labeled as cell cycle. On the left side of the axis, an arrow points to the first line labeled as cytokinesis and on the right end, there is a line labeled mitosis. Between these 2 spots are evenly spaced sections labeled G subscript 1, S, and G subscript 2. The vertical axis is labeled relative amount of D N A and has 3 equally spaced lines labeled as follows: 1 point 0 X, 1 point 5 X and 2 point 0 X.

35. In 1979, bones found outside Ekaterinburg, Russia, were shown to be those of Tsar Nicholas and his family, who were executed in 1918 by a Bolshevik firing squad in the Russian Revolution (see the introduction to Chapter 14). To prove that the skeletons were those of the royal family, mtDNA was extracted from the bone samples, amplified by PCR, and compared with mtDNA from living relatives of the tsar's family.
    a. Why was DNA from the mitochondria analyzed instead of nuclear DNA? What are some of the advantages of using

mtDNA for this type of study?

b. Mitochondrial DNA from which living relatives would provide useful information for verifying that the skeletons were those of the royal family?

36.

Antibiotics such as chloramphenicol, tetracycline, and erythromycin inhibit protein synthesis in bacteria but have no effect on the synthesis of proteins encoded by eukaryotic nuclear genes (see Section 15.4 in Chapter 15 for a discussion of the effects of antibiotics on protein synthesis). Cycloheximide inhibits the synthesis of proteins encoded by nuclear genes but has no effect on bacterial protein synthesis. How might these compounds be used to determine which proteins are encoded by mitochondrial and chloroplast genomes?

CHALLENGE QUESTIONS

Section 11.1

37. An explorer discovers a strange new species of plant and sends some of the plant tissue to a geneticist to study. The geneticist isolates chromatin from the plant and examines it with an electron microscope. She observes what appear to be beads on a string. She then adds a small amount of nuclease, which cleaves the string into individual beads that each contain 280 bp of

DNA. After digestion with more nuclease, a 120-bp fragment of DNA remains attached to a core of histone proteins. Analysis of the histone core reveals histones in the following proportions:

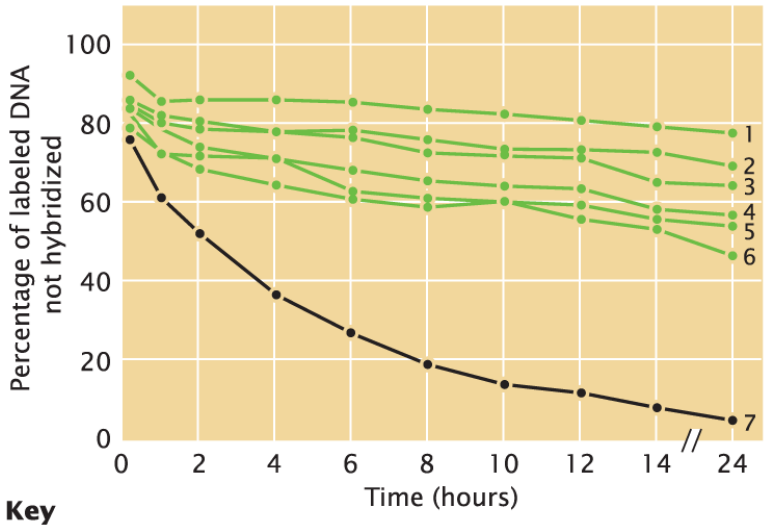
H1	12.5%
H2A	25%
H2B	25%
H3	0%
H4	25%
H7 (a new histone)	12.5%

On the basis of these observations, what conclusions could the geneticist make about the probable structure of the nucleosome in the chromatin of this plant?

Section 11.3

38.

In DNA-hybridization experiments on six species of plants in the genus *Vicia*, DNA was isolated from each of the six species, denatured by heating, and sheared into small fragments (W. Y. Chooi. 1971. *Genetics* 68:213–230). In one experiment, DNA from each species and from *E. coli* was allowed to renature. The graph shows the results of this renaturation experiment.



Key

1 = *V. melanops*, 2 = *V. sativa*, 3 = *V. benghalensis*,
4 = *V. atropurpurea*, 5 = *V. faba*, 6 = *V. narbonensis*, 7 = *E. coli*

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[Data from W. V. Choi. 1971. *Genetics* 68:213–230.]

Description

The horizontal axis represents time in hours ranging from 0 to 14 with an interval of 2. Then there is a break in the axis and the next point is 24 hours. The vertical axis shows percentage of labeled D N A not hybridized ranging from 0 to 100 with an interval of 20. The graph shows 7 lines, the first six lines represents species of Vicia as follows: 1, V melanops; 2, V sativa; 3, V benghalensis; 4, V atropurpurea; 5, V faba; and 6, V narbonensis. Line 7 represents E coli. The first 6 parallel lines representing Vicia species have a similar downward progression on the line graph. They all start between 80 and 95 percent and gradually decrease to around 50 to 80 percent. At 24 hours, line 1 ends at 79 percent, line 2 at 75 percent, line 3 at 65 percent, line 4 at 58 percent, line 5 at 55 percent, and line 6 at 48 percent. Line 7 is starts at 79 percent, falls steeply, and ends at 5 percent. All data are approximate.

Bob Gibbons/Alamy.

Fodder vetch (*Vicia sativa*).

a. Can you explain why the *E. coli* DNA renatures at a much faster rate than does DNA from any of the *Vicia* species?
b. Notice that for the *Vicia* species, the rate of renaturation is much faster in the first hour and then slows down. What might cause this initial rapid renaturation and the subsequent slowdown?

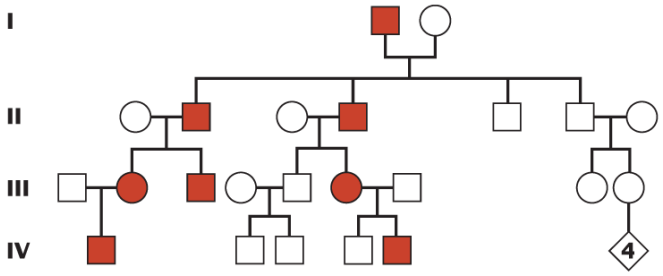
Section 11.4

39. DATA ANALYSIS Steven Frank and Laurence Hurst argued that a cytoplasmically inherited mutation in humans that has severe effects in males but no effect in females will not be eliminated from a population by natural selection because only females pass on mtDNA (S. A. Frank and L. D. Hurst. 1996. *Nature* 383:224). Using this argument, explain why males with Leber

hereditary optic neuropathy are more severely affected than females.

40. In a study of a muscle disorder, several affected families exhibited vision problems, muscle weakness, and deafness (M. Zeviani et al. 1990. *American Journal of Human Genetics* 47:904–914). Analysis of the mtDNA from affected members of these families revealed that large numbers of their mtDNA molecules possessed deletions of varying lengths. Different members of the same family and even different mitochondria from the same person possessed deletions of different sizes, so the underlying defect appeared to be a tendency for the mtDNA of affected persons to have deletions. A pedigree of one of the families studied is shown below (see Chapter 6 for a discussion of how to read and interpret pedigrees). The researchers concluded that this disorder is inherited as an autosomal dominant trait, and they mapped the disease-causing gene to a position on chromosome 10 in the nucleus.

    a. What characteristics of the pedigree rule out inheritance of a trait encoded by a gene in the mtDNA as the cause of this disorder?
    b. Explain how a mutation in a nuclear gene might lead to deletions in mtDNA.



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[Data from M. Zeviani et al. 1990. *American Journal of Human Genetics* 47:904–914.]

Description

In generation one, the male is affected and female is unaffected. They mate and have 2 affected sons and 2 unaffected sons in generation 2. One unaffected male from generation 2 mates with an unaffected female and has 2 unaffected daughters in generation 3. One affected male from generation 2 mates with an unaffected female and has 1 affected daughter and 1 unaffected son in generation 3. Another affected male from generation 2 mates with an unaffected female and has 1 affected son and 1 affected daughter in generation 3. The affected daughter of first affected male from generation2 mates with an unaffected male and has 1 affected son in generation 4. The unaffected son of second affected male from generation 2 mates with an unaffected female and has 2 unaffected sons in generation 4. The affected daughter of second affected male from generation 2 mates with an unaffected male and has 1 affected son and 1 unaffected son in generation 4. The unaffected daughter of unaffected male from generation 2 has 4 offspring of unidentified sex.

41. Mitochondrial DNA sequences have been detected in the nuclear genomes of many organisms, and cpDNA sequences are sometimes found in the mitochondrial genome. Propose a

mechanism for how such “promiscuous DNA” might move between nuclear, mitochondrial, and chloroplast genomes.

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 11.1

1. The Y chromosome contains large amounts of constitutive heterochromatin. Why might there be more constitutive heterochromatin in the Y chromosome than in other chromosomes? (Hint: See Evolution of the Y Chromosome in Chapter 4.)

2. Polytene chromosomes are giant chromosomes found in cells of the salivary glands of larval fruit flies and some other organisms. Polytene chromosomes arise when repeated rounds of replication occur without cell division, producing thousands of copies of DNA that lie side by side. Why do polytene chromosomes occur in the cells of salivary glands of larval fruit flies but not in other tissues? Propose some possible reasons. (If you are unfamiliar with the life cycle of fruit flies, see pp. A2–A3 in the Reference Guide to Model Genetic Organisms at the end of this book.)

Section 11.4

3. The Out of Africa hypothesis (also called the African replacement hypothesis) proposes that *Homo sapiens* arose in Africa and evolved there for several hundred thousand years. Then, some 85,000 years ago, a small band of *Homo sapiens* migrated out of Africa and populated the remainder of the world. Based on this hypothesis, what predictions would you make about worldwide human variation in mtDNA?

4. What are some possible social and ethical issues associated with mitochondrial replacement therapy (MRT)? Present arguments for and against approving MRT for prevention of the births of children with mitochondrial disorders.

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Create a concept map that summarizes the results of research on the relationship between telomere length and stress among children living in state-run orphanages in Romania. Include in your concept map how telomere length changes over cell divisions and the relationship between telomere length and stress.

Section 11.1

2. Outline the composition of eukaryotic chromatin by creating a concept map that includes the following terms: chromatin, protein, DNA, euchromatin, heterochromatin, histones, nonhistone proteins, facultative heterochromatin, constitutive heterochromatin, nucleosome, H1, H2A, H2B, H3, H4, linker DNA, protein octamer.

Section 11.3

3. Draw a concept map that explains the C-value paradox and the complexity of DNA sequences in eukaryotic genomes.

Section 11.4

4. Draw a concept map that summarizes the characteristics of organelle DNA by including the following terms: organelle DNA, mitochondrial DNA, chloroplast DNA, endosymbiotic theory, uniparental inheritance, replicative segregation, circular DNA, multiple copies of genome per cell, no histone proteins, small genome size .

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 12

DNA Replication and Recombination

Courtesy of Lana Stewart

Children with Meier-Gorlin syndrome have mutations in components of the origin-replication complex. This impairment most likely slows down cell division and results in a reduction in growth.

The Importance of a Good Start

When Ryan was born, it was immediately clear that he was special. Not only was he small for his gestational age, he also possessed small ears and had no kneecaps. A genetic specialist quickly recognized that Ryan exhibited a rare and peculiar genetic condition known as Meier-Gorlin syndrome, which results from a flaw in DNA replication.

Each eukaryotic chromosome contains an immense amount of DNA. A typical human chromosome consists of a single DNA molecule that is over a hundred million base pairs (bp) long. One of the challenges that eukaryotic cells face is copying this immense amount of genetic information whenever a cell divides. The process of eukaryotic replication takes place at breakneck speed—up to 5000 bp of DNA is copied every minute—but because of the immense size of eukaryotic chromosomes, replicating a typical human chromosome from a single starting point would require a week or more. Cells copy their DNA much more rapidly, often within minutes or hours. How is this accomplished?

The rapid replication in eukaryotic cells is achieved because replication does not initiate at a single point (which is what happens in bacteria) but instead starts at thousands of replication origins simultaneously. The use of many origins allows the entire eukaryotic genome to be replicated in a timely manner but creates another problem: how to ensure that the genome is precisely replicated once and only once per cell cycle, so that no genes go unreplicated and none are replicated multiple times. How does a

cell ensure that replication at thousands of origins is initiated precisely only once per cell cycle?

This precise copying of eukaryotic DNA is accomplished by separating the initiation of replication into two distinct steps. In the first step, the origins are *licensed*—approved for replication. This step takes place during the G_1 stage of the cell cycle, when licensing factors attach to each origin. In the second step, the replication machinery initiates replication at each licensed origin during the S stage. The key to this process is that the replication machinery functions only at licensed origins and that licensing occurs early in the cell cycle.

The importance of proper replication licensing is illustrated by children who have Meier-Gorlin syndrome. These children experience a reduction in growth during prenatal development. Consequently, they are born small and continue to grow slowly as children, ending up with short stature. Other traits include missing or undeveloped kneecaps and small ears. Many experience difficulty feeding in infancy and childhood but have normal intellectual ability and life span.

Meier-Gorlin syndrome is inherited as an autosomal recessive condition; most children with the disorder are born to parents who are heterozygous carriers and do not have the condition. When scientists examined the genomes of children with this condition, they discovered that most have mutations in one of several genes

that code for a multiprotein complex called the origin-replication complex, which serves to initiate replication by binding to replication origins and licensing them for replication. In children with Meier-Gorlin syndrome, the origin-replication complex is defective. This impairment most likely slows down cell division and results in growth retardation.

DNA replication, essential to all life, is a highly complex process in which dozens of proteins, enzymes, and DNA structures ensure that DNA is copied accurately and quickly. As illustrated by the Meier-Gorlin syndrome, defects in a single component of this system can disrupt the entire process and have major consequences.

THINK-PAIR-SHARE

- Defects in initiation of replication presumably slow down DNA synthesis and cell division, resulting in the growth reduction seen in children with Meier-Gorlin syndrome. However, most children with Meier-Gorlin syndrome also have small ears and missing kneecaps. How might flaws in replication initiation cause some body parts to be affected and not others? Can you propose some possibilities?

This chapter focuses on DNA replication, the process by which a cell doubles its DNA before division. We begin with the basic mechanism of replication that emerged from the DNA structure discovered by Watson and Crick. We then examine several different modes of replication, the requirements of replication, and the universal direction of DNA synthesis. We also examine the enzymes and proteins that participate in the process. Finally, we consider

the molecular details of recombination, which is closely related to replication and is essential for the segregation of homologous chromosomes, the production of genetic variation, and DNA repair.

12.1 Genetic Information Must Be Accurately Copied Every Time a Cell Divides

In a schoolyard game, a verbal message, such as “John’s brown dog ran away from home,” is whispered to a child, who runs to a second child and repeats the message. The message is relayed from child to child around the schoolyard until it returns to the original sender. Inevitably, the last child returns with an amazingly transformed message, such as “Joe Brown has a pig living under his porch.” The larger the number of children playing the game, the more garbled the message becomes. This game illustrates an important principle: errors arise whenever information is copied, and the more times it is copied, the greater the potential number of errors.

A complex multicellular organism faces a problem analogous to that of the children in the schoolyard game: how to faithfully transmit genetic instructions each time its cells divide. The solution to this problem is central to replication. A single-celled human zygote contains 6.4 billion base pairs of DNA; even a low rate of error during copying, such as once per million base pairs, would result in 6400 mistakes made every time a cell divided—errors that would be compounded at each of the millions of cell divisions that take place in human development.

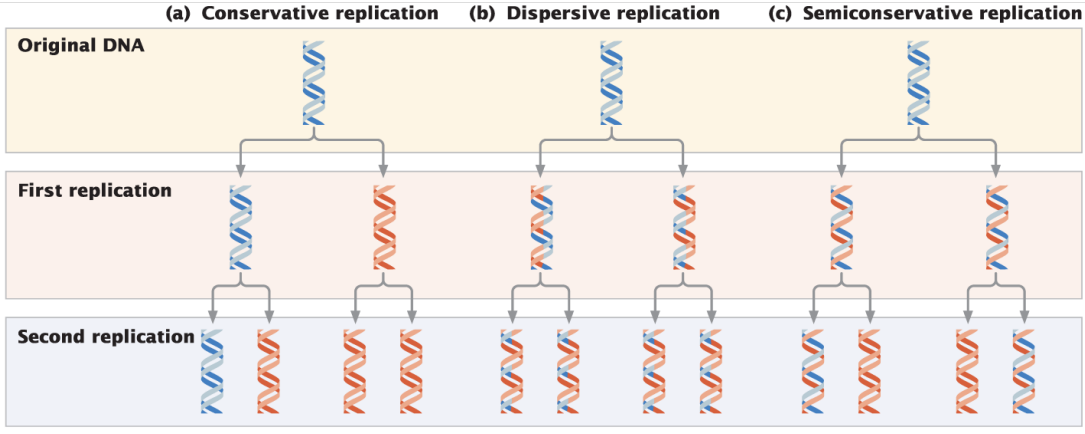
Not only must the copying of DNA be astoundingly accurate, it must also take place at rapid speed. The single circular chromosome of *Escherichia coli* contains about 4.6 million base pairs. At a rate of 1000 nucleotides per minute, replication of the entire chromosome would require over 3 days. Yet these bacteria are capable of dividing every 20 minutes. *E. coli* actually replicates its DNA at a rate of 1000 nucleotides per second, with less than one error in a billion nucleotides. How is this extraordinarily accurate and rapid process accomplished?

THINK-PAIR-SHARE Question 1

12.2 All DNA Replication Takes Place in a Semiconservative Manner

When Watson and Crick solved the three-dimensional structure of DNA in 1953 (see Figure 10.8), several important genetic implications were immediately apparent. The complementary nature of the two nucleotide strands in a DNA molecule suggested that during replication, each strand can serve as a template for the synthesis of a new strand. The specificity of base pairing (adenine with thymine, guanine with cytosine) implied that only one sequence of bases can be specified by each template strand, and so the two DNA molecules built on the pair of templates will be identical to the original. This process is called **semiconservative replication** because each of the original nucleotide strands remains intact (conserved), despite their no longer being combined in the same molecule; thus, the original DNA molecule is half (semi-) conserved during replication.

Initially, three models were proposed for DNA replication. In conservative replication (**Figure 12.1a**), the entire double-stranded DNA molecule serves as a template for a whole new molecule of DNA, and the original DNA molecule is *fully* conserved during replication. In dispersive replication (**Figure 12.1b**), both nucleotide strands break down (disperse) into fragments, which serve as templates for the synthesis of new DNA fragments, and then somehow reassemble into two complete DNA molecules. In this model, each resulting DNA molecule contains interspersed fragments of old and new DNA; none of the original molecule is conserved. Semiconservative replication (**Figure 12.1c**) is intermediate between these two models; the two nucleotide strands unwind, and each serves as a template for a new DNA molecule.



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12.1 Three proposed models of DNA replication: conservative replication, dispersive replication, and semiconservative replication. In this figure, the parental (original) DNA is colored blue, and newly synthesized DNA is colored red.

Description
Part a on the left shows conservative replication where original D N A helix is blue colored. First replication occurs and 1 red duplex and 1 blue duplex are formed below. Second replication occurs and blue duplex produces 1 red and 1 blue duplex while red duplex produces 2

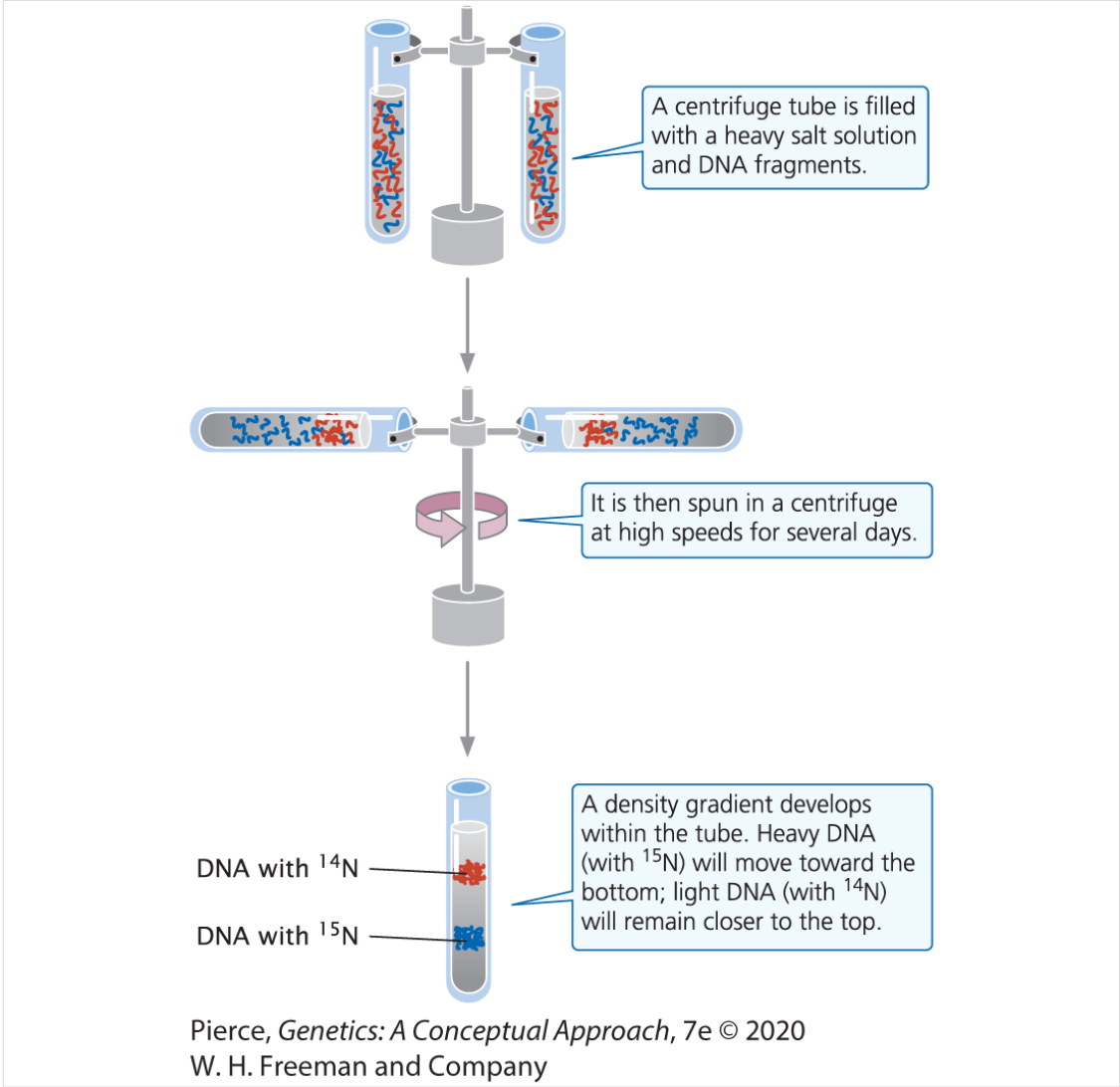
red duplexes below. Part b in the center shows dispersive replication where the original D N A strand is blue colored. First replication occurs and 2 D N A duplexes are produced below that are red-blue hybrid. Second replication occurs and each duplex produces 2 D N A duplexes below that are red-blue hybrid. Part c on the right shows semi conservative replication, where original D N A strand is blue colored. First replication occurs and 2 duplexes are produced below, each with a blue strand and a red strand. Second replication occurs and the following duplexes are produced below: 2 duplexes have a blue strand and a red strand each while 2 duplexes are completely red.

These three models allow different predictions to be made about the distribution of original DNA and newly synthesized DNA after replication. With conservative replication, after one round of replication, 50% of the molecules would consist entirely of the original DNA and 50% would consist entirely of new DNA. After a second round of replication, 25% of the molecules would consist entirely of the original DNA and 75% would consist entirely of new DNA. With each additional round of replication, the proportion of molecules with new DNA would increase, although the number of molecules with the original DNA would remain constant. Dispersive replication would always produce hybrid molecules, containing some original and some new DNA, but the proportion of new DNA within the molecules would increase with each replication event. In contrast, with semiconservative replication, one round of replication would produce two hybrid molecules, each consisting of half original DNA and half new DNA. After a second round of replication, half the molecules would be hybrid and the other half would consist of new DNA only. Additional rounds of replication would produce more and more molecules consisting entirely of new DNA, but a few hybrid molecules would persist.

Meselson and Stahl's Experiment

To determine which of the three models of replication applied to *E. coli* cells, Matthew Meselson and Franklin Stahl needed a way to distinguish old and new DNA. They accomplished this by using two isotopes of nitrogen, ^{14}N (the common form) and ^{15}N (a rare, heavy form). Meselson and Stahl grew a culture of *E. coli* in a medium that contained ^{15}N as the sole nitrogen source; after many generations, all the *E. coli* cells had ^{15}N incorporated into all the purine and pyrimidine bases of their DNA (see Figure 10.11). They took a sample of these bacteria, switched the rest of the bacteria to a medium that contained only ^{14}N, and then took additional samples of bacteria over the next few cellular generations. In each sample, the bacterial DNA that was synthesized before the change in medium contained ^{15}N and was relatively heavy, whereas any DNA synthesized after the switch contained ^{14}N and was relatively light.

Meselson and Stahl distinguished between the heavy ^{15}N-laden DNA and the light ^{14}N-containing DNA with the use of **equilibrium density gradient centrifugation** (**Figure 12.2**). In this technique, a centrifuge tube is filled with a heavy salt solution and a substance of unknown density—in this case, DNA fragments. The tube is then spun in a centrifuge at high speeds. After several days of spinning, a gradient of density develops within the tube, with high-density material at the bottom and low-density material at the top. The density of the DNA fragments matches that of the salt: light molecules rise and heavy molecules sink.



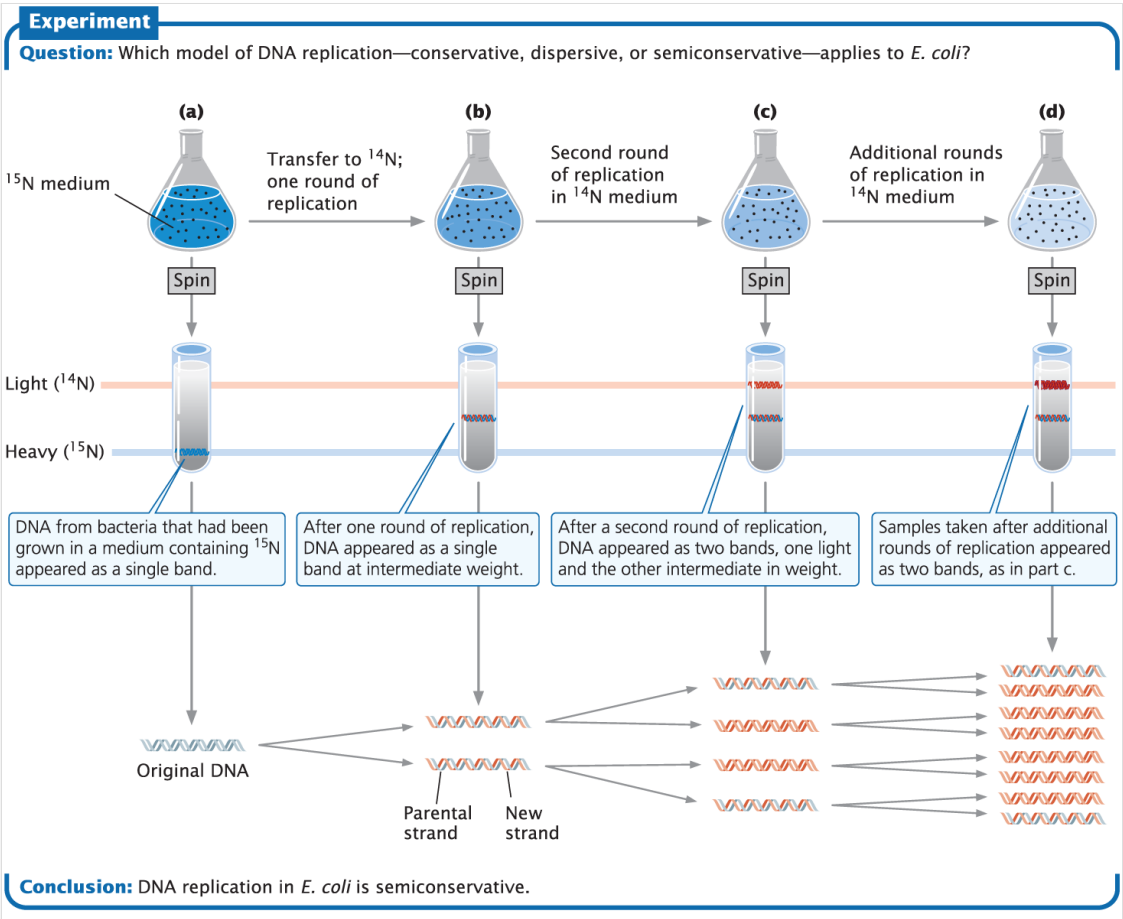
12.2 Meselson and Stahl used equilibrium density gradient centrifugation to distinguish between heavy ^{15}N-laden DNA and lighter ^{14}N-laden DNA.

Description
The illustration shows 2 tubes at the top containing red and blue colored D N A fragments. These tubes are positioned upright. A caption reads, a centrifuge tube is filled with a heavy salt solution and D N A fragments. These test tubes are now positioned horizontally in a centrifuge below and spun such that blue colored fragments move towards the test tube ends and red fragments move towards the test tube openings. A caption reads, It is then spun in a centrifuge at high speeds for several days. This leads to gradient density development. The illustration shows an upright test tube below with the red, lower-density, N labeled left superscript 14 D N A near the top and the blue, higher-density, N-labeled left superscript 15 D N A at the bottom. A caption reads, a density gradient develops within the tube. Heavy D N A (with N left superscript 15) will move toward the bottom; light D N A (with N left superscript 14) will remain closer to the top.

Meselson and Stahl found that DNA from bacteria grown only in a medium containing ^{15}N produced a single band at the position expected of DNA containing only ^{15}N (Figure 12.3a). DNA from bacteria transferred to the medium with ^{14}N and allowed one round of replication also produced a single band, but at a position intermediate between that expected of DNA containing only ^{15}N and that expected of

DNA containing only ^{14}N (Figure 12.3b). This result is inconsistent with the conservative replication model, which predicts one heavy band (the original DNA molecules) and one light band (the new DNA molecules). A single band of intermediate density is predicted by both the semiconservative and the dispersive models.

To distinguish between these two models, Meselson and Stahl grew the bacteria in a medium containing ^{14}N for a second generation. After a second round of replication in a medium with ^{14}N, two bands of equal intensity appeared, one in the intermediate position and the other at the position expected of DNA containing only ^{14}N (Figure 12.3c). All samples taken after additional rounds of replication produced the same two bands, and the band representing light DNA became progressively stronger (Figure 12.3d). Meselson and Stahl's results were exactly as expected for semiconservative replication and were incompatible with those predicted for both conservative and dispersive replication. **› TRY** PROBLEM 21



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12.3 Meselson and Stahl demonstrated that DNA replication is semiconservative.

Description

The illustration begins with a question that reads, Which model of D N A replication—conservative, dispersive, or semiconservative—applies to E. coli? The Part a on the extreme left shows bacteria grown in the heavy N left superscript 15 medium. A part of it is spun below and a single band appears on the N left superscript 15 at the bottom of the test tube. A caption reads, D N A from bacteria that had been grown on medium containing N left superscript 15 appeared as a single band. This leads to an original blue-colored D N A duplex below. Part b on the top left shows a part of bacteria from N left superscript 15 medium to N left superscript 14 medium that has undergone one round of replication. This is spun below and a band appears in the middle of the test tube between N left superscript 15 and N left superscript 14 band positions. A caption reads, after one round of replication, the D N A appeared as a single band at intermediate weight. This further leads to two duplexes with 1 blue strand and 1 red strand each. One of the D N A helixes has one parental strand and one new strand. Part c on the top right shows a part of bacteria after one round of replication that undergoes second round of replication. A part of this is spun and two bands are obtained below as follows: one light band towards N left superscript 14 and one intermediate band in the middle of test tube. A caption reads, after a second round of replication, D N A appeared as two bands, one light and the other intermediate in weight. This further leads to 2 red duplexes and 2 duplexes with one red strand and 1 blue strand each. Part d on the extreme right shows a part of bacteria after two rounds of replication that undergoes additional rounds of replication in N left superscript 14 medium. This is spun and 2 bands are obtained below: one light band towards N left superscript 14 and one intermediate band in the middle of test tube. A caption reads, samples taken after additional rounds of replication appeared as two bands, as in part c. This further leads to 8 duplexes below as follows: 6 red duplexes and 2 duplexes with one red strand and 1 blue strand each.

THINK-PAIR-SHARE Question 2

CONCEPTS

Meselson and Stahl convincingly demonstrated that replication in *E. coli* is semiconservative: each DNA strand serves as a template for the synthesis of a new DNA molecule.

✔ CONCEPT CHECK 1

How many bands of DNA would be expected in Meselson and Stahl's experiment after two rounds of *conservative* replication?

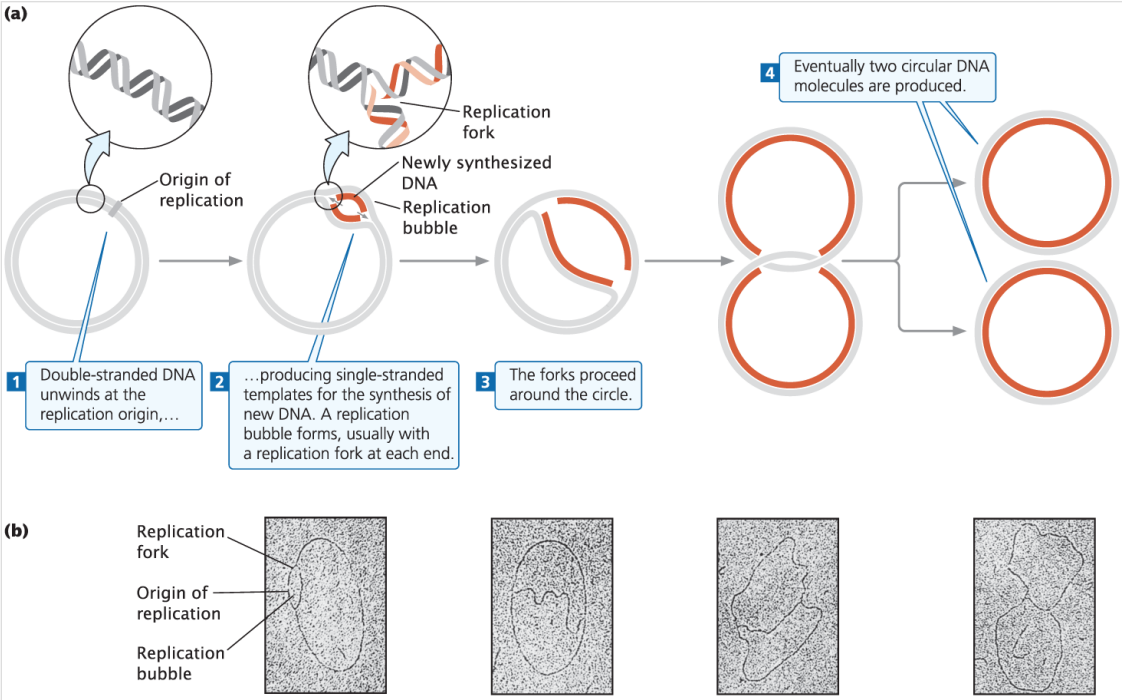
Modes of Replication

After Meselson and Stahl's work was published, investigators confirmed that other organisms also use semiconservative replication. No evidence was found for conservative or dispersive replication. There are, however, several different ways in which semiconservative replication can take place, differing principally in the nature of the template DNA—that is, whether it is linear or circular.

A segment of DNA that undergoes replication is called a **replicon**, and each replicon contains an **origin of replication**. Replication starts at the origin and continues until the entire replicon has been replicated. Bacterial chromosomes have a single origin of replication, whereas eukaryotic chromosomes contain many.

THETA REPLICATION

A common mode of replication that takes place in circular DNA, such as that found in *E. coli* and other bacteria, is called **theta replication** (**Figure 12.4a**) because it generates an intermediate structure that resembles the Greek letter theta (θ). In Figure 12.4a and all subsequent figures in this chapter, the original (template) strand of DNA is shown in gray and the newly synthesized strand of DNA is shown in red.



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12.4 Theta replication is a type of replication common in *E. coli* and other organisms possessing circular DNA. [Part b: Bernhard Hirt, L'Institut Suisse de Recherche Expérimentale sur le Cancer.]

Description

The illustration a at the top shows circular double-stranded D N A opening up at the origin of replication where a caption reads, double-stranded D N A unwinds at the replication origin. This leads to formation of a replication bubble to its right where new D N A strands are synthesized. The caption reads, producing single-stranded templates for the synthesis of the new D N A. A replication bubble forms, shown with a replication fork at each end. In the third stage, the forks extend throughout the circle. The caption reads, the forks proceed around the circle. In the fourth stage, two circular D N A strands are produced. The caption reads, eventually 2 circular D N A molecules are produced on the right. The electron microscopy images in part "b" below are as follows: The first photo on the extreme left shows a smaller circle formed along the line of a larger circle, the smaller circle being the replication bubble that appears at the start of replication at the origin of replication. The corner of the smaller bubble is labeled Replication fork and the outer line of the bubble is labeled origin of replication. The second and third photos on the right show the D N A strand with an organic theta symbol appearance, as the replication progresses. In the fourth photo, on the extreme right, there are 2 circular lines adjoined partially. A text below reads, Conclusion: the products of theta replication are 2 circular D N A molecules.

In theta replication, double-stranded DNA begins to unwind at the origin of replication, producing single nucleotide strands that then serve as templates on which new DNA can be synthesized. The

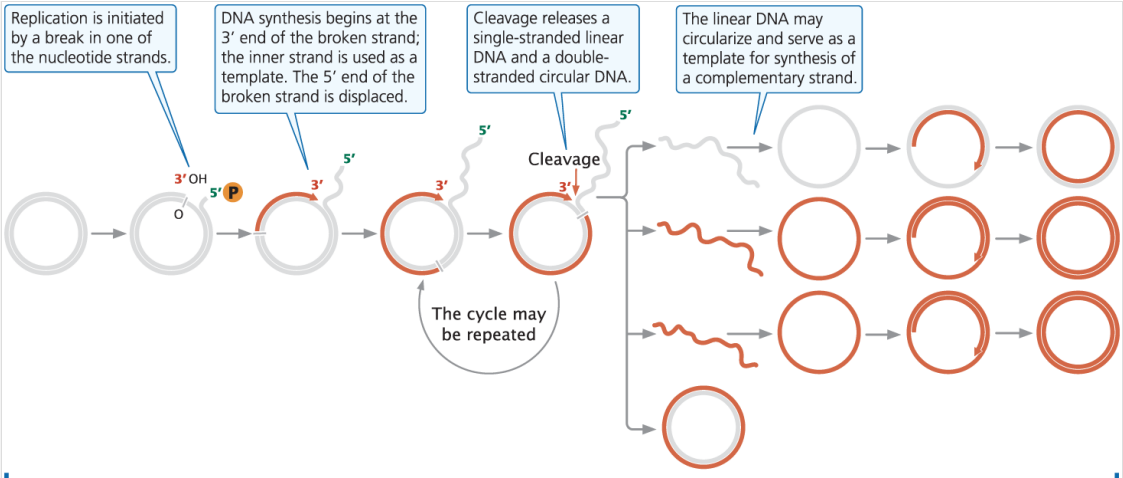
unwinding of the double helix generates a loop, termed a **replication bubble**. Unwinding may occur at one or both ends of the bubble, making it progressively larger. DNA replication on both of the template strands is simultaneous with unwinding. The point of unwinding, where the two strands separate from the double-stranded DNA helix, is called a **replication fork**.

If there are two replication forks, one at each end of the replication bubble, the forks proceed outward in both directions in a process called **bidirectional replication**, simultaneously unwinding and replicating the DNA until they eventually meet. If unidirectional replication with a single replication fork is present, it proceeds around the entire circle. Both bidirectional and unidirectional replication produce two complete circular DNA molecules, each consisting of one old and one new nucleotide strand.

John Cairns provided the first visible evidence of theta replication in 1963 by growing bacteria in the presence of radioactive nucleotides. After replication, each DNA molecule consisted of one "hot" (radioactive) strand and one "cold" (nonradioactive) strand. Cairns isolated DNA from the bacteria after replication, placed it on an electron-microscope grid, and then covered it with a photographic emulsion. Radioactivity present in the sample exposed the emulsion and produced a picture of the molecule (called an autoradiograph) in a manner similar to the light that exposes a photographic film. Because the newly synthesized DNA contained radioactive nucleotides, Cairns was able to produce electron micrographs of the replication process similar to those shown in **Figure 12.4b**.

ROLLING-CIRCLE REPLICATION

Another form of replication, called **rolling-circle replication** (**Figure 12.5**), takes place in some viruses and in the F factor of *E. coli* (a small circle of extrachromosomal DNA that controls mating, discussed in Section 9.3). This form of replication is initiated by a break in one of the nucleotide strands, which exposes a 3′-OH group and a 5′-phosphate group. New nucleotides are added to the 3′ end of the broken strand, using the inner (unbroken) strand as a template. As new nucleotides are added to the 3′ end, the 5′ end of the broken strand is displaced from the template, rolling out like thread being pulled off a spool. The 3′ end grows around the circle, giving rise to the name *rolling-circle replication*.



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12.5 Rolling-circle replication takes place in some viruses and in the F factor of *E. coli*.

Description

The illustration shows a gray colored circular D N A molecule on the left. This progresses to a break (labeled as O) in one of the nucleotide strand. The 3 prime end has an O H molecule and 5 prime has a P molecule. A caption reads, replication is initiated by a break in one of the nucleotide strands. D N A synthesis begins at 3 prime end. A caption reads, D N A synthesis begins at the 3 prime end of the broken strand; the inner strand is used as a template. The 5 prime end of the broken strand is displaced. The newly synthesized D N A strand on the right is red and increasing in length. A caption reads, cleavage releases a single-stranded linear D N A molecule from the double-stranded circular D N A. This stage can revert back to the previous stage and cycle may be repeated again. This leads to one original strand, two red strands, and one circular D N A with red strand on the outside, on the right side of the illustration. The original gray colored strand at the top right becomes circular and a complementary strand is formed around leading to a circular D N A strand. A caption reads, the linear D N A may circularize and serve as a template for the synthesis of a complementary strand. Both red colored strands eventually form one red circular D N A strand eventually. A caption reads, Conclusion: The products of rolling-circle replication are multiple circular D N A molecules.

The replication fork may continue around the circle a number of times, producing several linked copies of the same sequence. With each revolution around the circle, the growing 3′ end displaces the nucleotide strand synthesized in the preceding revolution. Eventually, the linear DNA molecule is cleaved from the circle, resulting in a double-stranded circular DNA molecule and a single-stranded linear DNA molecule. The linear molecule circularizes either before or after, serving as a template for the synthesis of a complementary strand.

LINEAR EUKARYOTIC REPLICATION

Circular DNA molecules that undergo theta or rolling-circle replication have a single origin of replication. Because of the limited size of these DNA molecules, replication starting from one origin can traverse the entire chromosome in a reasonable amount of time. The large linear chromosomes in eukaryotic cells, however, contain far too much DNA to be replicated speedily from a single origin. Eukaryotic replication proceeds at a rate ranging from 500 to 5000 nucleotides per minute at each

replication fork (considerably slower than bacterial replication). Even at 5000 nucleotides per minute at each fork, DNA synthesis starting from a single origin would require 7 days to replicate a typical human chromosome consisting of 100 million base pairs of DNA. The replication of eukaryotic chromosomes actually takes place in a matter of minutes or hours, not days. As discussed in the introduction to the chapter, this rapid rate is possible because replication is initiated at thousands of origins.

Typical eukaryotic replicons are from 20,000 to 300,000 base pairs in length (Table 12.1). At each origin of replication, the DNA unwinds and produces a replication bubble. Replication takes place on both strands at each end of the bubble, with the two replication forks spreading outward. Eventually, the replication forks of adjacent replicons run into each other, and the replicons fuse to form long stretches of newly synthesized DNA (Figure 12.6). Replication and fusion of all the replicons leads to two identical DNA molecules. Important features of theta replication, rolling-circle replication, and linear eukaryotic replication are summarized in Table 12.2. › **TRY** PROBLEM 22

TABLE 12.1 Number and length of replicons

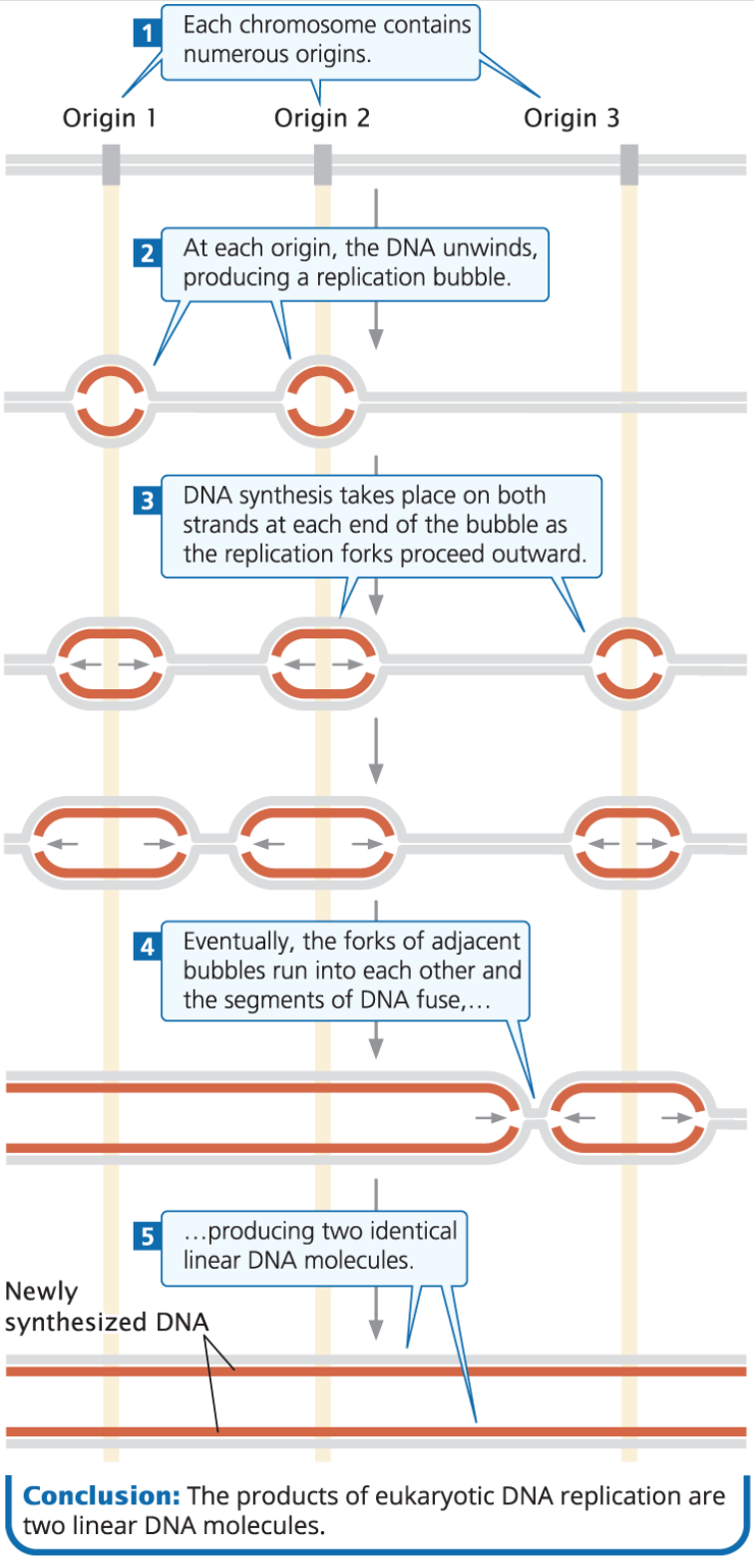
Organism	Number of Replication Origins	Average Length of Replicon (bp)
Escherichia coli (bacterium)	1	4,600,000
Saccharomyces cerevisiae (yeast)	500	40,000
Drosophila melanogaster (fruit fly)	3,500	40,000
Xenopus laevis (frog)	15,000	200,000
Mus musculus (mouse)	25,000	150,000

Data from B. L. Lewin, *Genes V* (Oxford: Oxford University Press, 1994), p. 536.

TABLE 12.2 Characteristics of theta, rolling-circle, and linear eukaryotic replication

Replication Model	DNA Template	Breakage of Nucleotide Strand	Number of Replicons	Unidirectional or Bidirectional	Products
Theta	Circular	No	1	Unidirectional or bidirectional	Two circular molecules

Rolling-circle	Circular	Yes	1	Unidirectional	One circular molecule and one linear molecule that may circularize
Linear eukaryotic	Linear	No	Many	Bidirectional	Two linear molecules



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12.6 Linear DNA replication takes place in eukaryotic chromosomes.

Description
The illustration shows different stages during the replication process, beginning with two linear D N A strands at the top. These linear D N As have 3 origins of replication: origin 1, origin 2, and origin 3. A caption reads, each chromosome contains numerous origins. In the next step below, the replication begins at origin 1 and origin 2 as the D N A unwinds and a semi circular replication bubble that is protruding outwards is formed. A caption reads, at each origin, the D N A unwinds, producing a replication bubble. In the next step below, the newly formed red D N A strands begin to increase in length as the replication bubble proceed outwards and a new replication bubble forms at origin 3. A caption reads, D N A synthesis takes place on both strands at each end of the bubble as the replication forks stretch outward. The newly formed D N A strands below keep increasing in size. A caption reads, eventually, the forks of adjacent bubbles run into each other and the segments of D N A fuse. The next step below shows the D N A strands newly synthesized at origin 1 and origin 2 fuse to become a linear D N A strand while the replication fork at origin C continues extending outwards. This leads to formation of 2 linear red colored, newly synthesized strands below for each of the older gray strands. A caption reads, producing two identical linear D N A molecules. A caption reads, Conclusion: The products of eukaryotic D N A replication are two linear D N A molecules.

CONCEPTS

Theta replication, rolling-circle replication, and linear eukaryotic replication differ with respect to the initiation and progression of replication, but all produce new DNA molecules by semiconservative replication.

✔ CONCEPT CHECK 2

Which type of replication requires a break in the nucleotide strand to get started?

a. Theta replication
b. Rolling-circle replication
c. Linear eukaryotic replication
d. All of the above

Requirements of Replication

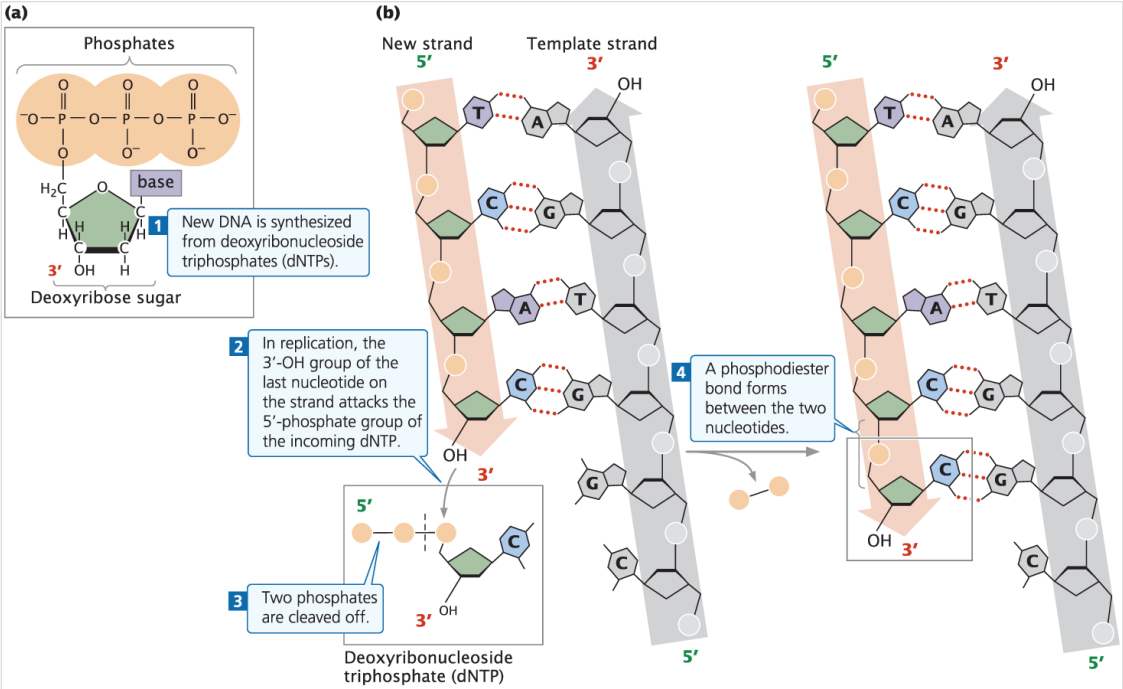
Although the process of replication includes many components, they can be combined into three major groups:

1. A template consisting of single-stranded DNA
2. Raw materials (substrates) to be assembled into a new nucleotide strand
3. Enzymes and other proteins that “read” the template and assemble the substrates into a DNA molecule

Because of the semiconservative nature of DNA replication, a double-stranded DNA molecule must unwind to expose the bases that act as a template for the assembly of new polynucleotide strands, which will be complementary and antiparallel to the template strands.

The raw materials from which new DNA molecules are synthesized are deoxyribonucleoside triphosphates (dNTPs), each consisting of a deoxyribose sugar and a base (a nucleoside) attached to

three phosphate groups (Figure 12.7a). In DNA synthesis, nucleotides are added to the 3′-OH group of the growing nucleotide strand (Figure 12.7b). The 3′-OH group of the last nucleotide on the strand attacks the 5′-phosphate group of the incoming dNTP. Two phosphate groups are cleaved from the incoming dNTP, and a phosphodiester bond is created between the two nucleotides.



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12.7 New DNA is synthesized from deoxyribonucleoside triphosphates (dNTPs). The newly synthesized strand is complementary and antiparallel to the template strand. The two strands are held together by hydrogen bonds (represented by red dotted lines) between the bases.

Description

Part a on the left shows a deoxyribonucleoside triphosphate. This molecule has a pentose sugar where C 1 and C 5 is bonded to a methylene group which is further bonded to 3 phosphate molecules. A caption reads, new D N A is synthesized from deoxyribonucleoside triphosphates or d N T Ps. Part b in the center shows a red colored newly synthesized strand forming from 5 prime to 3 prime to a grey colored template strand that runs from 5 prime to 3 prime. A d N T P containing the cytosine base being added to the ongoing strand and forming triple bond with a nucleotide containing guanine on the opposing strand. Similarly, T is double bonded to A. The nucleotides T, C, A, G are bonded to A, G, T, G respectively. A caption reads, In replication, the 3 prime O H group of the last nucleotide on the strand attacks the 5 prime phosphate group of the incoming d N T P. An illustration shows 2 magnified phosphate groups cleaving off from the d N T P. A caption reads, two phosphates are cleaved off. A new phosphodiester bond is formed between the two nucleotides. Part c on the right shows one more d N T P added to the newly synthesized strand. A caption reads, a phosphodiester bond forms between the 2 nucleotides.

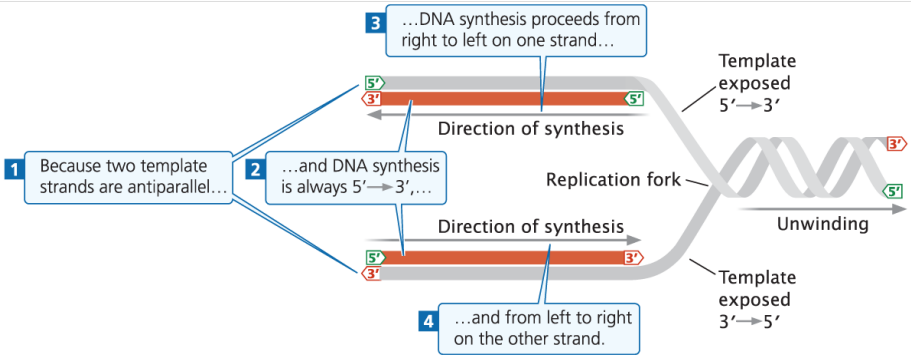
DNA synthesis does not happen spontaneously. Rather, it requires a host of enzymes and proteins that function in a coordinated manner. We examine this complex array of proteins and enzymes as we consider the replication process in more detail.

CONCEPTS

DNA synthesis requires a single-stranded DNA template, deoxyribonucleoside triphosphates, a growing nucleotide strand, and a group of enzymes and proteins.

Direction of Replication

In DNA synthesis, new nucleotides are joined one at a time to the 3′ end of the newly synthesized strand. **DNA polymerases**, the enzymes that synthesize DNA, can add nucleotides only to the 3′ end of the growing strand (not the 5′ end), so new DNA strands always elongate in the same 5′-to-3′ direction (5′ → 3′). Because the two single-stranded DNA templates are antiparallel and strand elongation is always 5′ → 3′, if synthesis on one template proceeds from, say, right to left, then synthesis on the other template must proceed in the opposite direction, from left to right (**Figure 12.8**). As DNA unwinds during replication, the antiparallel nature of the two DNA strands means that one template is exposed in the 5′ → 3′ direction and the other template is exposed in the 3′ → 5′ direction. So how can synthesis take place simultaneously on both strands at the replication fork?



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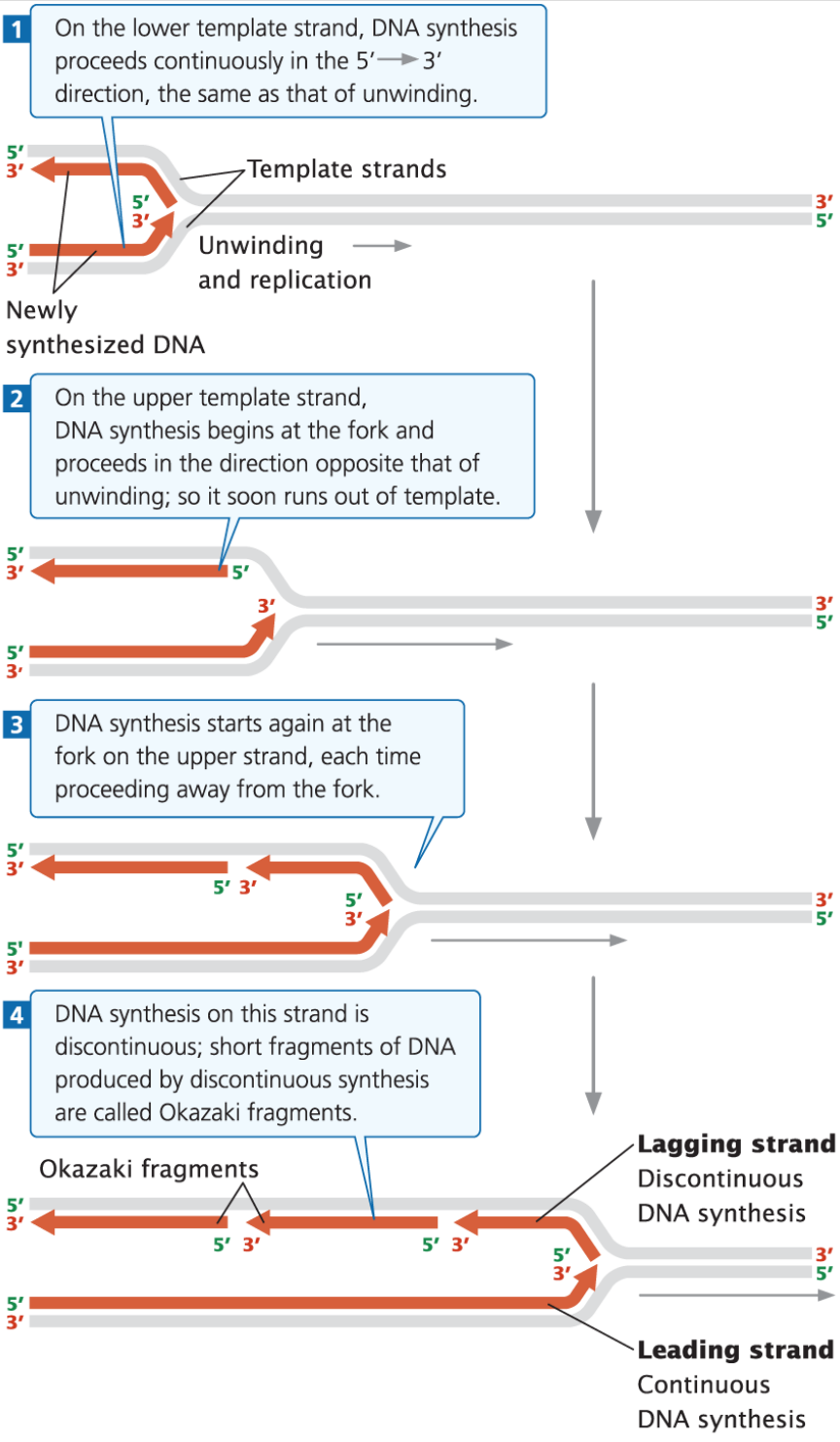
12.8 DNA synthesis takes place in opposite directions on the two DNA template strands. DNA replication at a single replication fork begins when a double-stranded DNA molecule unwinds to provide two single-stranded templates.

Description
The illustration shows a D N A molecule. The D N A strands are unwinding from left to right. A replication fork is present at the point where unwinding initiates. The template strands are antiparallel after unwinding. A caption reads, because two template strands are antiparallel and D N A synthesis is always 5 prime to 3 prime. For the top template strand that runs from 5 prime to 3 prime, the D N A synthesis occurs in opposite direction (right to left). A caption reads, D N A synthesis proceeds from right to left on one strand. For the bottom template strand that runs from 3 prime to 5 prime, the D N A synthesis occurs in opposite direction (left to right). A caption reads, and from left to right on the other strand.

CONTINUOUS AND DISCONTINUOUS REPLICATION

As the DNA unwinds, the template strand that is exposed in the 3′ → 5′ direction (the lower strand in Figures 12.8 and 12.9) allows the new strand to be synthesized continuously, in the 5′ → 3′ direction. This new strand, which undergoes **continuous replication**, is called the **leading strand**.

The other template strand is exposed in the 5′ → 3′ direction (the upper strand in Figures 12.8 and 12.9). After a short length of the DNA has been unwound, synthesis must proceed 5′ → 3′, that is, in the direction *opposite* that of unwinding (**Figure 12.9**). Because only a short length of DNA needs to be unwound before synthesis on this strand gets started, the replication machinery soon runs out of template. By that time, more DNA has unwound, providing a new template at the 5′ end of the new strand. DNA synthesis must start anew at the replication fork and proceed in the direction opposite that of the movement of the fork until it runs into the previously replicated segment of DNA. This process is repeated again and again, so synthesis of this strand is in short, discontinuous bursts. The newly made strand that undergoes **discontinuous replication** is called the **lagging strand**.



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12.9 DNA synthesis is continuous on one template strand of DNA and discontinuous on the other.

Description

The illustration shows a grey colored D N A double strand, at the top, that is unwinding from left to right. For the top D N A strand running from 5 prime to 3 prime, a red colored D N A strand is synthesized from light to left. For the bottom D N A strand running from 3 prime to 5 prime, a red colored D N A strand is synthesized from left to right. A caption reads, on the lower template strand, D N A synthesis proceeds continuously in the 5 prime to 3 prime direction, the same as that of unwinding. In the next step below, the newly synthesized D N A strand for the top D N A strand runs out of template. A caption reads, on the upper template strand, D N A synthesis begins at the fork and proceeds in the direction opposite that of unwinding; so it soon runs out of template. As the unwinding proceeds, another new strand begins from right to left at the top template strand. A caption reads, D N A synthesis starts again at the fork on the upper strand, each time proceeding away from the fork. The unwinding proceeds further to a third D N A strand below, known as the lagging strand, which starts from right to left on the top template strand. The newly synthesized discontinuous D N A strands on the top template strand are called Okazaki fragments and the single newly synthesized continuous strand at the bottom template strand is called the leading strand. A caption reads, D N A synthesis on this strand is discontinuous; short fragments of D N A produced by discontinuous synthesis are called Okazaki fragments.

OKAZAKI FRAGMENTS

The short lengths of DNA produced by discontinuous replication of the lagging strand are called **Okazaki fragments**, after Reiji Okazaki, who discovered them. In bacterial cells, each Okazaki fragment ranges from about 1000 to 2000 nucleotides in length; in eukaryotic cells, they are about 100 to 200 nucleotides long. Okazaki fragments on the lagging strand are linked together to create a continuous new DNA molecule. To see how replication occurs continuously on one strand and discontinuously on the other, view (A) **Animation 12.1**.

CONCEPTS

All DNA synthesis is 5′ → 3′, meaning that new nucleotides are always added to the 3′ end of the growing nucleotide strand. At each replication fork, synthesis of the leading strand proceeds continuously and that of the lagging strand proceeds discontinuously.

✔ CONCEPT CHECK 3

Discontinuous replication is a result of which property of DNA?

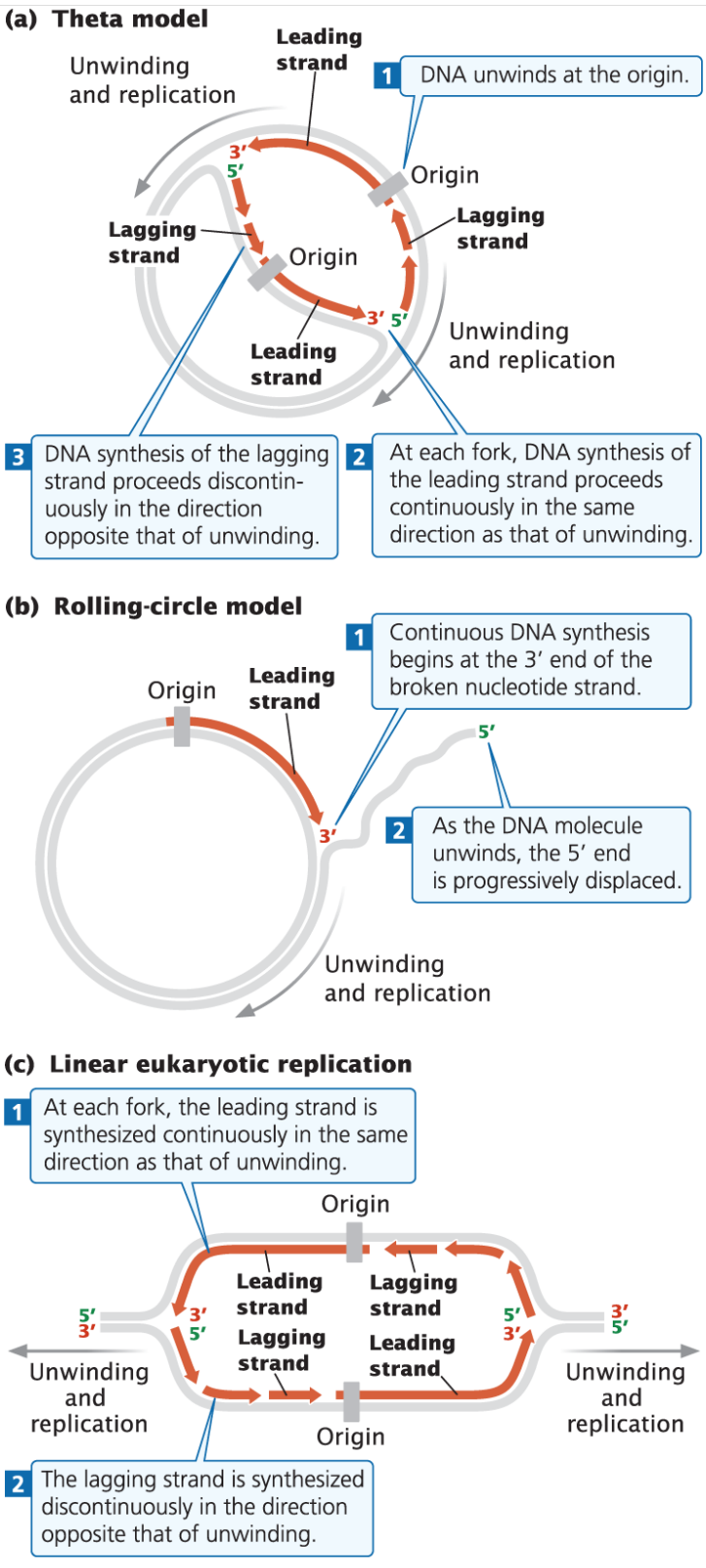
a. Complementary bases
b. Charged phosphate group
c. Antiparallel nucleotide strands
d. Five-carbon sugar

CONNECTING CONCEPTS CONNECT

The Direction of Synthesis in Different Modes of Replication

Let's relate the direction of DNA synthesis to the modes of replication examined earlier. In theta replication (**Figure 12.10a**), the DNA unwinds at one particular location, the origin, and a replication bubble is formed. If the bubble has two replication forks, one at each end, synthesis takes place simultaneously at both forks (bidirectional replication). At each fork, synthesis on one of the template strands proceeds in the same direction as that of unwinding; this newly replicated strand is the leading strand and is replicated continuously. On the other template strand, synthesis proceeds in the direction opposite that of unwinding; this newly synthesized strand is the lagging strand and is replicated discontinuously. Focus on just one of the template strands within the bubble. Notice that synthesis on this template strand is continuous at one

fork but discontinuous at the other. This difference arises because DNA synthesis is always in the same direction (5′ → 3′), but the two forks are moving in opposite directions.



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12.10 The process of replication differs in theta replication, rolling-circle replication, and linear eukaryotic replication.

Description
Part a at the top shows theta replication. Circular D N A double strands unwinds at the origin on both the strands. A caption reads, D N A unwinds at the origin. The unwinding and replication occur in both directions clockwise and anticlockwise from the origin. For the outer strand, the discontinuous lagging strand is synthesized anticlockwise and the continuous leading strand is also synthesized in the same direction (5 prime to 3 prime). A caption reads, at each fork, D N A synthesis of the leading strand proceeds continuously in the same direction as that of unwinding. For the inner strand, the continuous leading strand and discontinuous lagging strand are also anticlockwise. A caption reads, D N A synthesis of the lagging strand proceeds discontinuously in the direction opposite that of unwinding. Part b below shows rolling circle model. Circular D N A double strands unwinds at the origin in a clockwise direction. A continuous leading strand is synthesized in clockwise direction. A caption reads, continuous D N A synthesis begins at the 3 prime end of the broken nucleotide strand. As the leading strand is synthesized, the 5 prime end of the outer original D N A strand is displaced from its 5 prime end. A caption reads, as the D N A molecule unwinds, the 5 prime end is progressively displaced. Part c below shows linear eukaryotic replication. A double stranded D N A molecule unwinds in both left and right directions as a replication bubble is formed. The top strand, discontinuous lagging strand, and continuous leading strand are synthesized from right to left (from 5 prime to 3 prime). A caption reads, at each fork, the leading strand is synthesized continuously in the same direction as that of unwinding. The bottom strand, discontinuous lagging strand, and continuous leading strand are synthesized from left to right (from 5 prime to 3 prime). A caption reads, the lagging strand is synthesized discontinuously in the direction opposite that of unwinding.

Rolling-circle replication (**Figure 12.10b**) is somewhat different because there is no replication bubble. Replication begins at the 3′ end of the broken nucleotide strand. Continuous replication takes place on the circular template as new nucleotides are added to this 3′ end.

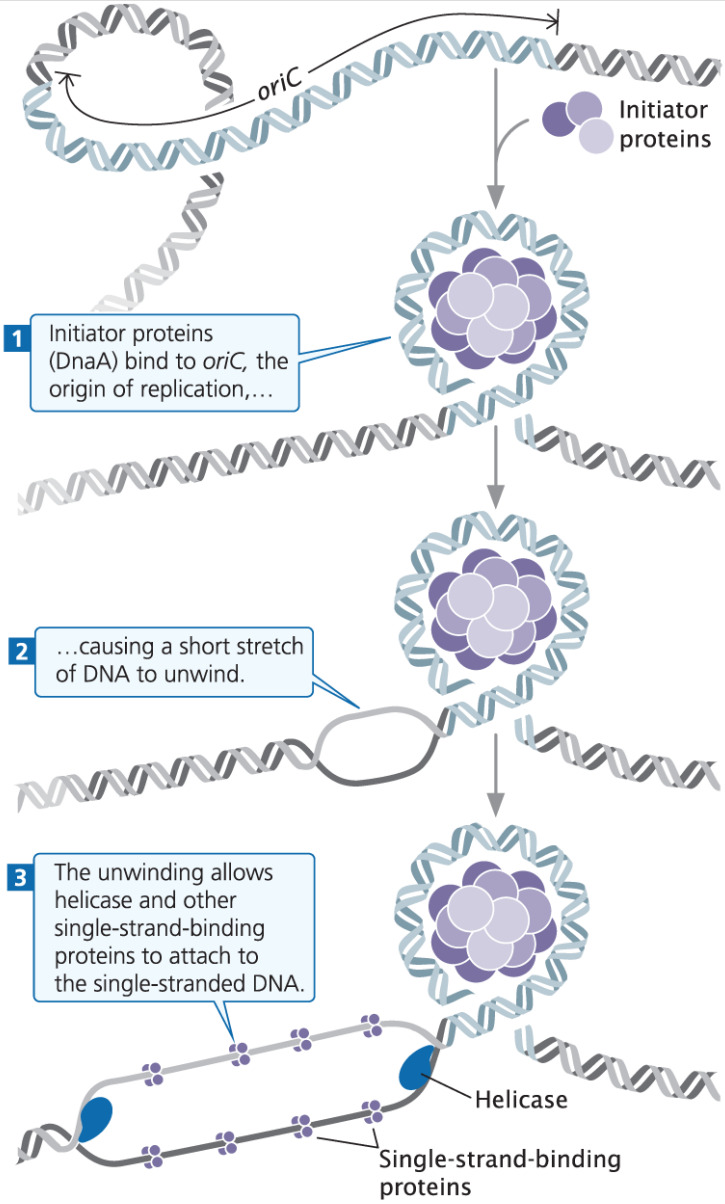
The replication of linear molecules of DNA, such as those found in eukaryotic cells, produces a series of replication bubbles (**Figure 12.10c**). DNA synthesis in these bubbles is the same as that in the single replication bubble of theta replication; it begins at the center of each replication bubble and proceeds at two forks, one at each end of the bubble. At both forks, synthesis of the leading strand proceeds in the same direction as that of unwinding, whereas synthesis of the lagging strand proceeds in the direction opposite that of unwinding. ❯ **TRY PROBLEM 24a–c**

12.3 Bacterial Replication Requires a Large Number of Enzymes and Proteins

Replication takes place in four stages: initiation, unwinding, elongation, and termination. The following discussion of the process of replication will focus on bacterial systems, in which replication has been most thoroughly studied and is best understood. Although many aspects of replication in eukaryotic cells are similar to those in bacterial cells, there are some important differences. We compare bacterial replication with eukaryotic and archaeal replication in Section 12.4.

Initiation

The circular chromosome of *E. coli* has a single origin of replication (*oriC*). The minimal sequence required for *oriC* to function consists of 245 bp that contain several critical sites. **Initiator proteins** (known as DnaA in *E. coli*) bind to *oriC* and cause a short section of DNA to unwind. This unwinding allows helicase and other single-strand-binding proteins to attach to the polynucleotide strand (**Figure 12.11**).



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12.11 *E. coli* DNA replication begins when initiator proteins bind to *oriC*, the origin of replication.

Description

The illustration shows a stretch of D N A duplex, at the top, with a part in middle labeled o r i C. This stretch of D N A loops around initiator proteins. A caption reads, initiator proteins (D N A A) bind to O r i C, the origin of replication, causing a short stretch of D N A to unwind. In the next step below, two helicase proteins attach at a replication fork site each.

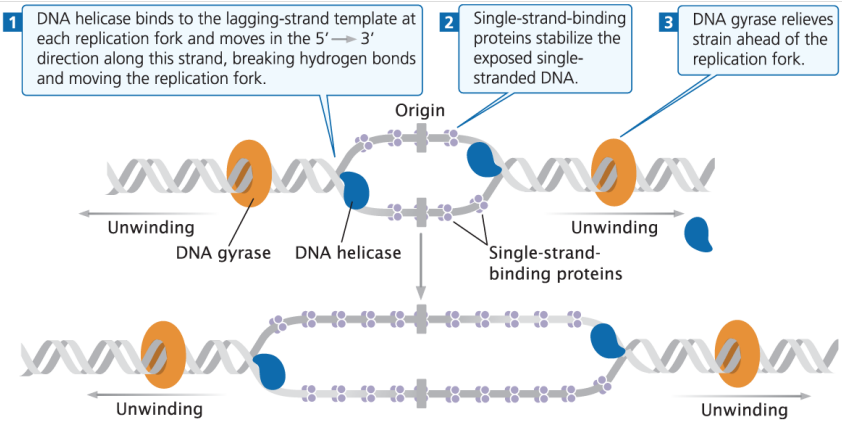
The template strands formed below have multiple clusters of single strand binding proteins attached to them. A caption reads, the unwinding allows helicase and other single-strand-binding proteins to attach to the single-stranded D N A.

Unwinding

DNA synthesis requires a single-stranded template, and therefore double-stranded DNA must be unwound before DNA synthesis can take place. The cell relies on several proteins and enzymes to accomplish the unwinding.

DNA HELICASE

DNA helicase breaks the hydrogen bonds that exist between the bases of the two nucleotide strands of a DNA molecule. In bacteria, helicase cannot *initiate* the unwinding of double-stranded DNA; the initiator protein first separates DNA strands at the origin, providing a short stretch of single-stranded DNA. Initiator protein, loader proteins, and other accessary proteins then recruit and load helicase onto the single-stranded DNA at each replication fork. Once bound to single-stranded DNA, helicase moves in the 5′→3′ direction along this strand, thus also moving the replication fork (**Figure 12.12**).



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12.12 DNA helicase unwinds DNA by binding to the lagging-strand template at each replication fork and moving in the 5′→3′ direction.

Description

The illustration shows a D N A double strand at the top where two helicase proteins are attached to one replication fork each. Three single strand binding proteins are attached to each of the template strands flanking the origin. The D N A is unwinding in both directions outwards from the replication forks formed. On both sides of the helicase proteins, one D N A gyrase molecule is encircling the original D N A double strand. A caption reads, D N A helicase binds to the lagging strand template at each replication fork and moves in the 5 prime to 3 prime direction along this strand, breaking hydrogen bonds and moving the replication fork. Single-strand-binding proteins stabilize the exposed single-stranded D N A. D N A gyrase relieves strain ahead of the replication fork. In the next step below, more single strand binding proteins are attached to each of the strands as the unwinding of the original D N A double strand continues.

SINGLE-STRAND-BINDING PROTEINS

After DNA has been unwound by helicase, **single-strand-binding proteins (SSBs)** attach tightly to the exposed single-stranded DNA (see Figure 12.12). These proteins protect the single-stranded

nucleotide chains and prevent the formation of secondary structures such as hairpins (see Figure 10.18) that would interfere with replication. Unlike many DNA-binding proteins, SSBs are indifferent to base sequence: they will bind to any single-stranded DNA. Single-strand-binding proteins form tetramers (groups of four); each tetramer covers from 35 to 65 nucleotides.

DNA GYRASE

Another protein essential for the unwinding process is the enzyme **DNA gyrase**, a topoisomerase. As discussed in Chapter 11 and the introduction to this chapter, topoisomerases control the supercoiling of DNA. They come in two major types: type I topoisomerases alter supercoiling by making single-strand breaks in DNA, while type II topoisomerases create double-strand breaks. DNA gyrase is a type II topoisomerase. In replication, it reduces the torsional strain (torque) that builds up ahead of the replication fork as a result of unwinding (see Figure 12.12). It reduces torque by making a double-strand break in one segment of the DNA helix, passing another segment of the helix through the break, and then resealing the broken ends of the DNA. This action, which requires ATP, removes a twist in the DNA and reduces the supercoiling.

A group of antibiotics called 4-quinolones kill bacteria by binding to DNA gyrase and inhibiting its action. The inhibition of DNA gyrase results in the cessation of DNA synthesis and bacterial growth. An example of a 4-quinolone is nalidixic acid, which was first

introduced in the 1960s and is commonly used to treat urinary infections. Many bacteria have acquired resistance to quinolones through mutations in the gene for DNA gyrase.

CONCEPTS

Replication is initiated at an origin of replication, where an initiator protein binds and causes a short stretch of DNA to unwind. DNA helicase breaks hydrogen bonds at a replication fork, and single-strand-binding proteins stabilize the separated strands. DNA gyrase reduces the torsional strain that develops as the two strands of double-helical DNA unwind.

✔ CONCEPT CHECK 4

Place the following components in the order in which they are first used in the course of replication: helicase, single-strand-binding protein, DNA gyrase, initiator protein.

Elongation

In the elongation stage of replication, single-stranded DNA is used as a template for the synthesis of DNA. This process requires a series of enzymes.

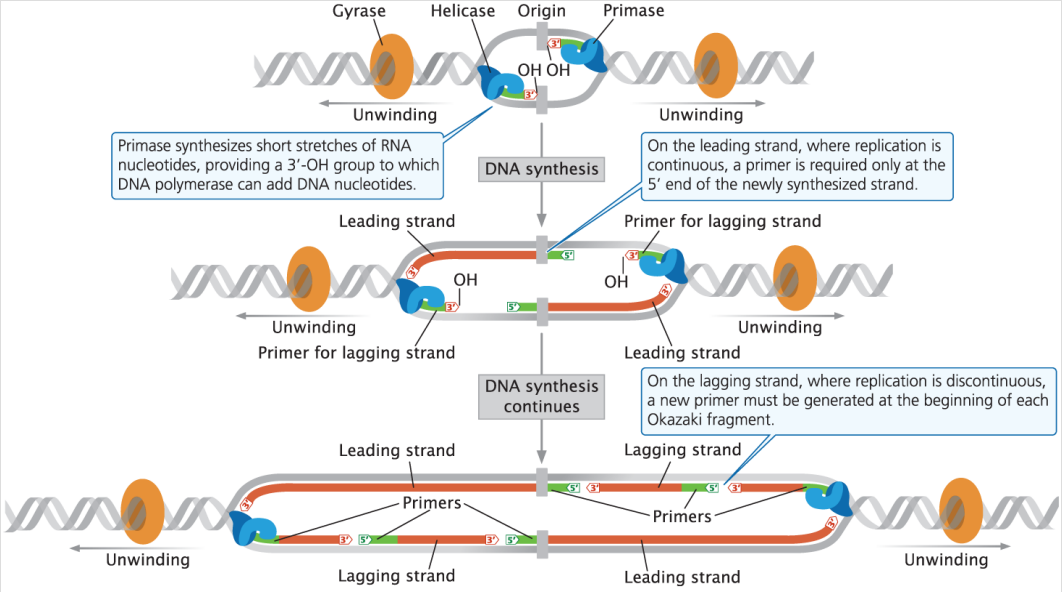
THE SYNTHESIS OF PRIMERS

All DNA polymerases require a nucleotide with a 3′-OH group to which a new nucleotide can be added. Because of this requirement,

DNA polymerases cannot initiate DNA synthesis on a bare template; rather, they require an existing 3′-OH group to get started. How, then, does DNA synthesis begin?

An enzyme called **primase** synthesizes short stretches (about 10–12 nucleotides long) of RNA nucleotides, or **primers**, which provide a 3′-OH group to which DNA polymerases can attach DNA nucleotides. (Because primase is an RNA polymerase, it does not require a preexisting 3′-OH group to start synthesis of a nucleotide strand.) All newly synthesized DNA molecules have short RNA primers embedded within them; these primers are later removed and replaced with DNA nucleotides.

On the leading strand at a replication fork, where DNA synthesis is continuous, a primer is required only at the 5′ end of the newly synthesized strand. On the lagging strand, where replication is discontinuous, a new primer must be generated at the beginning of each Okazaki fragment (**Figure 12.13**). Primase forms a complex with helicase at the replication fork and moves along the template of the lagging strand. The single primer on the leading strand is probably synthesized by the primase–helicase complex on the template of the lagging strand of the *other* replication fork, at the opposite end of the replication bubble. ❯ **TRY PROBLEM 29**



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12.13 Primase synthesizes short stretches of RNA nucleotides, providing a 3′-OH group to which DNA polymerase can add DNA nucleotides.

Description

The illustration shows a D N A double strand, at the top, where two helicase proteins are attached to one replication fork each. The D N A is unwinding in both directions outwards from the replication forks formed. On both sides of the helicase proteins, one D N A gyrase molecule is encircling the original D N A double strand. A primase with a 3 prime O H group attaches to each of the helicase protein. A caption reads, primase synthesizes short stretches of R N A nucleotides, providing a 3 prime O H group to which D N A polymerase can add D N A nucleotides. D N A synthesis occurs below and a continuous leading strand is synthesized on the top strand and another continuous leading strand is synthesized on the bottom strand. A primer for lagging strand is bound to each of the helicase protein. A caption reads, on the leading strand, where replication is continuous, a primer is required only at the 5 prime end of the newly synthesized strand. The D N A synthesis continues below and the unwinding progresses. Two lagging strands are synthesized on each template strands while the leading strands continue to increase in length. A new primer is generated at the start of each Okazaki fragment. The unwinding continues and a caption reads, on the lagging strand, where replication is discontinuous, a new primer must be generated at the beginning of each Okazaki fragment.

CONCEPTS

Primase synthesizes a short stretch of RNA nucleotides (a primer), which provides a 3′-OH group for the attachment of DNA nucleotides to start DNA synthesis.

✔ CONCEPT CHECK 5

Primers are synthesized where on the lagging strand?

a. Only at the 5′ end of the newly synthesized strand
b. Only at the 3′ end of the newly synthesized strand
c. At the beginning of every Okazaki fragment
d. At multiple places within an Okazaki fragment

DNA SYNTHESIS BY DNA POLYMERASES

After DNA has unwound and a primer has been added, DNA polymerases elongate the new polynucleotide strand by catalyzing DNA polymerization. The best-studied polymerases are those of *E. coli*, which has at least five different DNA polymerases. Two of them, DNA polymerase I and DNA polymerase III, carry out DNA synthesis in replication; the other three have specialized functions in DNA repair (Table 12.3).

TABLE 12.3 Characteristics of DNA polymerases in *E. coli*

DNA Polymerase	5′→3′ Polymerase Activity	3′→5′ Exonuclease Activity	5′→3′ Exonuclease Activity	Function
I	Yes	Yes	Yes	Removes and replaces primers

II	Yes	Yes	No	DNA repair; restarts replication after damaged DNA halts synthesis
III	Yes	Yes	No	Elongates DNA
IV	Yes	No	No	DNA repair
V	Yes	No	No	DNA repair; translesion DNA synthesis

DNA polymerase III is a large multiprotein complex that acts as the main workhorse of replication. DNA polymerase III synthesizes DNA by adding new nucleotides to the 3′ end of a growing DNA strand. This enzyme has two enzymatic activities (see Table 12.3). Its 5′→3′ polymerase activity allows it to add new nucleotides in the 5′→3′ direction. Its 3′→5′ exonuclease activity allows it to remove nucleotides in the 3′→5′ direction, enabling it to correct errors. If a nucleotide with an incorrect base is inserted into the growing DNA strand, DNA polymerase III uses its 3′→5′ exonuclease activity to back up and remove the incorrect nucleotide. It then resumes its 5′→3′ polymerase activity. These two functions together allow DNA polymerase III to efficiently and accurately synthesize new DNA molecules. DNA polymerase III has high *processivity*, which means that it is capable of adding many nucleotides to the growing DNA strand without releasing the template: it normally holds on to the template and continues synthesizing DNA until the template has been completely replicated. The high processivity of DNA polymerase III is ensured by one of the polypeptides that constitutes the enzyme. This ring-shaped polypeptide, termed the ***β* sliding**

clamp, encircles the DNA and allows the polymerase to slide easily along the DNA template strand during replication. DNA polymerase III adds DNA nucleotides to the primer, synthesizing the DNA of both the leading and the lagging strands.

The first *E. coli* polymerase to be discovered, **DNA polymerase I**, also has 5′ → 3′ polymerase and 3′ → 5′ exonuclease activities (see Table 12.3), which allow the enzyme to synthesize DNA and to correct errors. Unlike DNA polymerase III, however, DNA polymerase I also possesses 5′ → 3′ exonuclease activity, which is used to remove the primers laid down by primase and replace them with DNA nucleotides by synthesizing in a 5′ → 3′ direction (see Figure 12.14). After DNA polymerase III has initiated synthesis at a primer and moved downstream, DNA polymerase I removes the RNA nucleotides of the primer, replacing them with DNA nucleotides. DNA polymerase I has lower processivity than does DNA polymerase III. The removal and replacement of primers appears to constitute the main function of DNA polymerase I. DNA polymerases II, IV, and V function in DNA repair.

Despite their differences, all of *E. coli*'s DNA polymerases

- synthesize any sequence specified by the template strand;
- synthesize in the 5′ → 3′ direction by adding nucleotides to a 3′-OH group;
- use dNTPs to synthesize new DNA;
- require a 3′-OH group to initiate synthesis;

- catalyze the formation of a phosphodiester bond by joining the 5′-phosphate group of the incoming nucleotide to the 3′-OH group of the preceding nucleotide on the growing strand, cleaving off two phosphates in the process;
- produce newly synthesized strands that are complementary and antiparallel to the template strands;
- are associated with a number of other proteins. **› TRY PROBLEM 26**

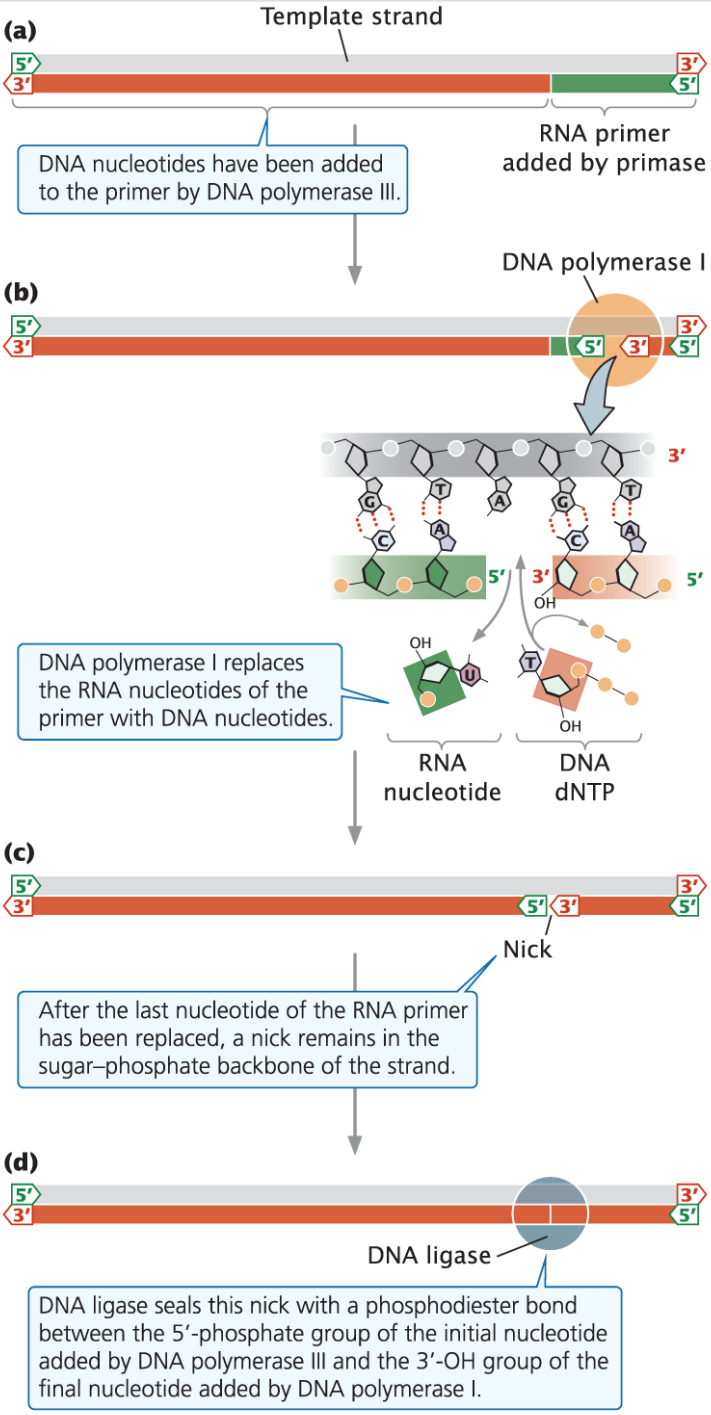
CONCEPTS

DNA polymerases synthesize DNA in the 5′ → 3′ direction by adding new nucleotides to the 3′ end of a growing nucleotide strand.

PRIMER REPLACEMENT AND DNA LIGASE

After DNA polymerase III attaches a DNA nucleotide to the 3′-OH group on the last nucleotide of the RNA primer, each new DNA nucleotide then provides the 3′-OH group needed for the next DNA nucleotide to be added. This process continues as long as a template is available (Figure 12.14a). DNA polymerase I follows DNA polymerase III and, using its 5′ → 3′ exonuclease activity, removes the RNA primer. It then uses its 5′ → 3′ polymerase activity to replace the RNA nucleotides with DNA nucleotides. DNA polymerase I attaches the first nucleotide to the OH group at the 3′ end of the preceding Okazaki fragment and then continues, in the 5′ → 3′ direction along

the nucleotide strand, removing and replacing, one at a time, the RNA nucleotides of the primer (Figure 12.14b).



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12.14 DNA ligase seals the nick left by DNA polymerase I in the sugar–phosphate backbone.

Description
Part a, at the top, shows a template D N A strand running from 5 prime to 3 prime. Below it is a newly synthesized D N A strand and towards the right side is a R N A primer added by primase. A caption reads, D N A nucleotides have been added to the primer by D N A polymerase 3. Part b below shows a round D N A polymerase 1 molecule attached to the D N A double strand at the site of R N A primer. An illustration shows the magnified view of polymerase replacing R N A nucleotides with the D N A nucleotides (d N T P). A caption reads, D N A polymerase 1 replaces the R N A nucleotide of the primer with D N A nucleotides. Part c below shows D N A template strand and below it; a larger newly synthesized strand and one shorter newly synthesized strand. The 3 prime end of shorter newly synthesized strand is adjacent to the 5 prime end of the larger strand with a small nick between them. A caption reads, After the last nucleotide of the R N A primer has been replaced, a nick remains in the sugar-phosphate backbone of the strand. Part d below shows a D N A ligase molecule encircling the strands at the site of the nick. A caption reads, D N A ligase seals this nick with a phosphodiester bond between the 5 prime P group of the initial nucleotide added by D N A polymerase 3 and the 3 prime O H group of the final nucleotide added by D N A polymerase 1.

After polymerase I has replaced the last nucleotide of the RNA primer with a DNA nucleotide, a break (or nick) remains in the sugar–phosphate backbone of the new DNA strand because the 3′-OH group of the last nucleotide to have been added by DNA polymerase I is not attached to the 5′-phosphate group of the first nucleotide added by DNA polymerase III (**Figure 12.14c**). This nick is sealed by the enzyme **DNA ligase**, which catalyzes the formation of a phosphodiester bond without adding another nucleotide to the

strand (Figure 12.14d). Some of the major enzymes and proteins required for bacterial DNA replication are summarized in Table 12.4.

TABLE 12.4 Components required for replication in bacterial cells

Component	Function
Initiator protein	Binds to origin and separates strands of DNA to initiate replication
DNA helicase	Unwinds DNA at replication fork
Single-strand-binding proteins	Attach to single-stranded DNA and prevent secondary structures from forming
DNA gyrase	Moves ahead of the replication fork, making and resealing breaks in the double-helical DNA to release the torque that builds up as a result of unwinding at the replication fork
DNA primase	Synthesizes a short RNA primer to provide a 3′-OH group for the attachment of DNA nucleotides
DNA polymerase	Elongates a new nucleotide strand from the 3′-OH group provided by the primer

III	
DNA polymerase I	Removes RNA primers and replaces them with DNA
DNA ligase	Joins Okazaki fragments by sealing breaks in the sugar–phosphate backbone of newly synthesized DNA

CONCEPTS

After primers have been removed and replaced, the nick in the sugar–phosphate backbone of the new DNA strand is sealed by DNA ligase.

✔ CONCEPT CHECK 6

Which bacterial enzyme removes the primers?

a. Primase
b. DNA polymerase I
c. DNA polymerase III
d. Ligase

ELONGATION AT THE REPLICATION FORK

Now that the major enzymatic components of elongation—DNA polymerases, helicase, primase, and ligase—have been introduced, let's consider how these components interact at the replication fork.

Because the synthesis of the leading and lagging strands takes place simultaneously, two units of DNA polymerase III must be present at the replication fork, one for each strand. These two units of DNA polymerase III are connected; the lagging-strand template loops around so that it is in position for 5′ → 3′ replication. In this way, the DNA polymerase III complex is able to carry out 5′ → 3′ replication simultaneously on both templates, even though they run in opposite directions. See how replication takes place on both strands simultaneously by viewing **Animation 12.2**.

In summary, each active replication fork requires five basic components:

1. Helicase to unwind the DNA
2. Single-strand-binding proteins to protect the single nucleotide strands and prevent secondary structures
3. DNA gyrase to remove strain ahead of the replication fork
4. Primase to synthesize primers with a 3′-OH group at the beginning of each DNA fragment
5. DNA polymerase to synthesize the leading and lagging nucleotide strands

You can see how the different components of the replication process work together by viewing **Animation 12.3** and **Animation 12.4**.

Termination

In some DNA molecules, replication is terminated whenever two replication forks meet. In others, specific termination sequences (called *Ter* sites) block further replication. A termination protein, called Tus in *E. coli*, binds to these sequences, creating a Tus-*Ter* complex that blocks the movement of helicase, thus stalling the replication fork and preventing further DNA replication. Each Tus-*Ter* complex blocks a replication fork moving in one direction but not the other.

The Fidelity of DNA Replication

Overall, the error rate in replication is less than one mistake per billion nucleotides. How is this incredible accuracy achieved?

DNA polymerases are very particular in pairing nucleotides with their complements on the template strand. Errors in nucleotide selection by DNA polymerase arise only about once per 100,000 nucleotides. Most of the errors that do arise in nucleotide selection are corrected in a second process called **proofreading**. When a DNA polymerase inserts an incorrect nucleotide into the growing strand, the 3′-OH group of the mispaired nucleotide is not correctly positioned in the active site of the DNA polymerase for accepting the next nucleotide. The incorrect positioning stalls the polymerization reaction, and the 3′ → 5′ exonuclease activity of DNA polymerase removes the incorrectly paired nucleotide. DNA polymerase then

inserts the correct nucleotide. Together, proofreading and nucleotide selection result in an error rate of only one in 10 million nucleotides.

A third process, called **mismatch repair** (discussed further in Chapter 18), corrects errors after replication is complete. Any incorrectly paired nucleotides remaining after replication produce a deformity in the secondary structure of the DNA; that deformity is recognized by enzymes, which excise the incorrectly paired nucleotide and use the original nucleotide strand as a template to replace the incorrect nucleotide. Mismatch repair thus requires the ability to distinguish between the old and the new strands of DNA because the enzymes need some way of determining which of the two incorrectly paired bases to remove. In *E. coli*, methyl groups (—CH_3) are added to particular nucleotide sequences but only *after* replication. Thus, immediately after DNA synthesis, only the old DNA strand is methylated. It can therefore be distinguished from the newly synthesized strand, and mismatch repair takes place preferentially on the unmethylated nucleotide strand. No single process could produce this level of accuracy; a series of processes are required, each process catching errors missed by the preceding ones.

THINK-PAIR-SHARE Question 3

CONCEPTS

DNA replication is extremely accurate, with less than one error per billion nucleotides. The high level of accuracy in DNA replication is produced by precise nucleotide selection, proofreading, and mismatch repair.

✔ CONCEPT CHECK 7

Which mechanism requires the ability to distinguish between newly synthesized and template strands of DNA?

a. Nucleotide selection
b. DNA proofreading
c. Mismatch repair
d. All of the above

CONNECTING CONCEPTS CONNECT

The Basic Rules of Replication

Bacterial DNA replication requires a number of enzymes (see Table 12.4), proteins, and DNA sequences that function together to synthesize a new DNA molecule. These components are important, but we must not become so immersed in the details of the process that we lose sight of the general principles of replication.

1. Replication is always semiconservative.
2. Replication begins at sequences called origins of replication.
3. DNA synthesis begins with the synthesis of short segments of RNA called primers.
4. The elongation of DNA strands is always in the 5′ → 3′ direction.
5. New DNA is synthesized from dNTPs; in the polymerization of DNA, two phosphate groups are cleaved from a dNTP, and the resulting nucleotide is added to the 3′-OH group of the growing nucleotide strand.
6. Replication is continuous on the leading strand and discontinuous on the lagging strand.
7. New nucleotide strands are complementary and antiparallel to their template strands.
8. Replication takes place at very high rates and is astonishingly accurate, thanks to precise nucleotide selection, proofreading, and mismatch repair.

12.4 Eukaryotic DNA Replication Is Similar to Bacterial Replication but Differs in Several Aspects

Although eukaryotic replication resembles bacterial replication in many respects, replication in eukaryotic cells presents several additional challenges. First, the much greater size of eukaryotic genomes requires that replication be initiated at multiple origins. Second, eukaryotic chromosomes are linear, whereas prokaryotic chromosomes are circular. Third, the DNA template is associated with histone proteins in the form of nucleosomes, and nucleosome assembly must immediately follow DNA replication.

Eukaryotic Origins of Replication

Researchers first isolated eukaryotic origins of replication from yeast cells by demonstrating that certain DNA sequences confer the ability to replicate when transferred from a yeast chromosome to small circular pieces of DNA (plasmids). These **autonomously replicating sequences (ARSs)** enabled any DNA they were attached to to replicate. They were subsequently shown to be the origins of replication in yeast chromosomes. The nature of origins of replication in many multicellular eukaryotes are still poorly understood. Origins in different eukaryotic organisms vary greatly in sequence, although they usually contain a number of A–T base pairs. Evidence suggests that eukaryotic origins are often defined more by modifications to chromatin structure (epigenetic modifications, see Chapter 21) than by specific sequences. A multiprotein complex, the **origin-recognition**

complex (ORC), serves as the initiator by binding to defined origins and initiating replication. In multicellular eukaryotes, there are often many more potential replication origins than are actually used during DNA synthesis. Whether the sites that are used are determined at random or are somehow specified is not known.

Another difference in replication of eukaryotes involves the role of the initiator. In bacteria, helicase is unable to bind to double-stranded DNA; initiator protein first melts DNA, creating a single-stranded template that helicase then binds to. In eukaryotes, however, the initiator ORC recruits and loads helicase onto double-stranded DNA at the origin. This takes place during G_1 of the cell cycle. Then during S phase, helicase becomes activated and begins separating double-stranded DNA into single-stranded templates that are used in DNA synthesis.

CONCEPTS

Eukaryotic DNA contains many origins of replication. At each origin, a multiprotein origin-recognition complex binds to double-stranded DNA and recruits helicase to the origin.

✔ CONCEPT CHECK 8

In comparison with prokaryotes, what are some differences in the genome structure of eukaryotic cells that affect how replication takes place?

DNA Synthesis and the Cell Cycle

In rapidly dividing bacteria, DNA replication is continuous. In eukaryotic cells, however, replication is coordinated with the cell cycle. Passage

through the cell cycle, including the onset of replication, is controlled by cell cycle checkpoints. The important G_1/S checkpoint (see Section 2.2) holds the cell in G_1 until the DNA is ready to be replicated. After the G_1/S checkpoint is passed, the cell enters S phase and the DNA is replicated. A replication licensing system ensures that the DNA is not replicated again until after the cell has passed through mitosis.

The Licensing of DNA Replication

The use of thousands of origins of replication in eukaryotes requires that the entire genome must be replicated once, and only once, in each cell cycle.

As discussed in the chapter introduction, initiation of DNA replication in eukaryotes is divided into two distinct steps. In the first step, the origins are *licensed*—approved for replication. This step takes place early in the cell cycle, when **replication licensing factors** attach to each origin. In the second step, the replication machinery initiates replication at each licensed origin. The key is that the replication machinery functions only at licensed origins and that licensing occurs early in the cell cycle.

Licensing occurs in G_1 of interphase when the origin-recognition complex binds to an origin. ORC, with the help of two additional licensing factors, allows a complex called MCM2-7 (for minichromosome maintenance) to bind to an origin. Then, in S phase, the MCM2-7 complex associates with several cofactors and forms an active helicase that unwinds double-stranded DNA for replication. After replication has begun, several

mechanisms prevent MCM2-7 from binding to DNA and reinitiating replication at the origins until after mitosis has been completed.

Unwinding

Helicases that separate double-stranded DNA have been isolated from eukaryotic cells, as have single-strand-binding proteins and topoisomerases (which have a function equivalent to the DNA gyrase in bacterial cells). These enzymes and proteins are assumed to function in unwinding eukaryotic DNA in much the same way as their bacterial counterparts do.

Topoisomerase enzymes are also required to remove supercoiling that builds up ahead of the replication fork in eukaryotes, just as DNA gyrase does in bacteria. Topoisomerase enzymes remove the supercoils by clamping tightly to the DNA and breaking one or both of its strands. The strands then revolve around each other, removing the supercoiling and the strain. After the DNA has relaxed, the topoisomerase reseals the broken ends of the DNA strands. Some anticancer drugs work by interfering with these topoisomerases. Camptothecin compounds, isolated from the happy tree *Camptotheca acuminate*, insert themselves into the DNA gap created by topoisomerase I and block the topoisomerase from resealing the broken ends of DNA. This poisons the topoisomerase so that it is unable to remove supercoils ahead of replication. Accumulating supercoils halts the replication machinery and prevents the proliferation of cancer cells. But like many other anticancer drugs, camptothecin also inhibits the replication of normal, noncancerous cells, which is why chemotherapy makes many patients sick.

THINK-PAIR-SHARE Question 4

Eukaryotic DNA Polymerases

Some significant differences between the processes of bacterial and eukaryotic replication are in the number and functions of DNA polymerases. Eukaryotic cells contain many more different DNA polymerases than bacteria do, which function in replication, recombination, and DNA repair.

Three DNA polymerases carry out most of nuclear DNA synthesis during replication: DNA polymerase α, DNA polymerase δ, and DNA polymerase ε (Table 12.5). **DNA polymerase α** has primase activity and initiates nuclear DNA synthesis by synthesizing an RNA primer, followed by a short string of DNA nucleotides. After DNA polymerase α has laid down from 30 to 40 nucleotides, **DNA polymerase δ** completes replication on the lagging strand. Similar in structure and function to DNA polymerase δ, **DNA polymerase ε** replicates the leading strand. Other DNA polymerases take part in repair and recombination or catalyze the replication of organelle DNA.

TABLE 12.5 DNA polymerases in eukaryotic cells

DNA Polymerase	5′→3′ Polymerase Activity	3′→5′ Exonuclease Activity	Cellular Function
α (alpha)	Yes	No	Initiation of

			nuclear DNA synthesis and DNA repair; has primase activity
δ (delta)	Yes	Yes	Lagging-strand synthesis of nuclear DNA, DNA repair, and translesion DNA synthesis
ε (epsilon)	Yes	Yes	Leading-strand synthesis
γ (gamma)	Yes	Yes	Replication and repair of mitochondrial DNA
ζ (zeta)	Yes	No	Translesion DNA synthesis
η (eta)	Yes	No	Translesion DNA synthesis
θ (theta)	Yes	No	DNA repair

ι (iota)	Yes	No	Translesion DNA synthesis
κ (kappa)	Yes	No	Translesion DNA synthesis
λ (lambda)	Yes	No	DNA repair
μ (mu)	Yes	No	DNA repair
σ (sigma)	Yes	No	Nuclear DNA replication (possibly), DNA repair, and sister-chromatid cohesion
ϕ (phi)	Yes	No	Translesion DNA synthesis
Rev1	Yes	No	DNA repair

Note: The three polymerases listed at the top of the table are those that carry out nuclear DNA replication.

Some DNA polymerases, such as DNA polymerase δ and DNA polymerase ε, are capable of replicating DNA at high speed and with high fidelity (few mistakes) because they have active sites that snugly and exclusively accommodate the four normal DNA nucleotides: adenosine, guanosine, cytidine, and thymidine monophosphates. As a result of this specificity, distorted DNA templates and abnormal bases are not readily

accommodated within the active site of the enzyme. When these errors are encountered in the DNA template, the high-fidelity DNA polymerases stall and are unable to bypass the lesion.

Other DNA polymerases have lower fidelity but are able to bypass distortions in the DNA template. These specialized **translesion DNA polymerases** generally have a more open active site and are able to accommodate and copy templates with abnormal bases, distorted structures, and bulky lesions. Thus, these specialized enzymes can bypass such errors, but because their active sites are more open and accommodating, they tend to make more errors. In replication, high-speed, high-fidelity enzymes are generally used until they encounter a replication block. At that point, one or more of the translesion DNA polymerases takes over, bypasses the lesion, and continues replicating a short section of DNA. Then the translesion polymerases detach from the replication fork, and high-fidelity polymerases resume replication with high speed and accuracy. DNA-repair enzymes often repair the errors produced by the translesion polymerases, although some of these errors may escape detection and lead to mutations.

THINK-PAIR-SHARE Question 5

CONCEPTS

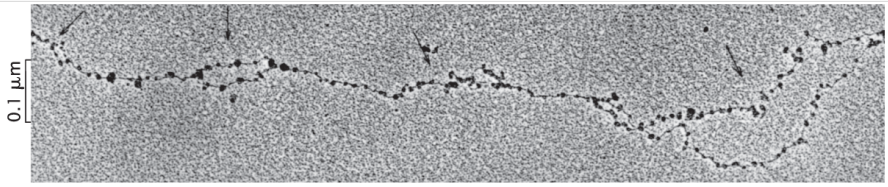
There are a large number of different DNA polymerases in eukaryotic cells. DNA polymerases α, δ, and ε carry out replication on the leading and lagging strands. Other DNA polymerases carry out DNA repair or synthesize organelle DNA. Specialized translesion polymerases are used to bypass distortions of the DNA template that normally stall the main DNA polymerases.

✔ CONCEPT CHECK 9

Some of the eukaryotic DNA polymerases have a tendency to make errors in replication. Why would a cell use an error-prone DNA polymerase instead of one that is more accurate?

Nucleosome Assembly

As we have seen, eukaryotic DNA is complexed with histone proteins to form nucleosomes, structures that contribute to the stability and packing of DNA (see Figure 11.4). In replication, chromatin structure is disrupted by the replication fork, but nucleosomes are quickly reassembled on the two new DNA molecules. Electron micrographs of eukaryotic DNA, such as that in **Figure 12.15**, show that recently replicated DNA is already covered with nucleosomes.



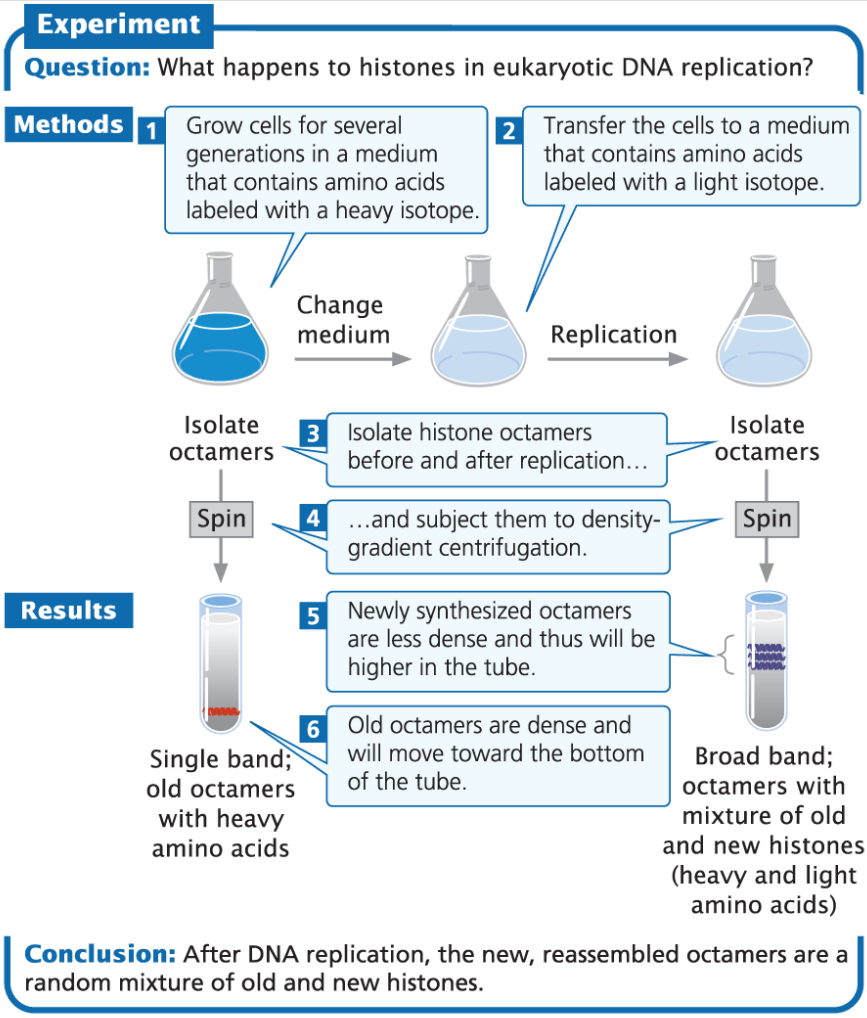
Victoria Foe.

12.15 Nucleosomes are quickly reassembled on newly synthesized DNA. This electron micrograph of eukaryotic DNA in the process of replication clearly shows that newly replicated DNA is already covered with nucleosomes (dark circles).

The creation of new nucleosomes requires three steps: (1) the disruption of the original nucleosomes on the parental DNA molecule ahead of the replication fork; (2) the redistribution of preexisting histones on the new DNA molecules; and (3) the addition of newly synthesized histones to complete the formation of new nucleosomes. Before replication, a single DNA molecule is associated with histone proteins. After replication and nucleosome assembly, two DNA molecules are associated with histone

proteins. Do the original histones of a nucleosome remain together, attached to one of the new DNA molecules, or do they disassemble and mix with new histones on both DNA molecules?

Techniques similar to those employed by Meselson and Stahl to determine the mode of DNA replication were used to address this question. Cells were cultivated for several generations in a medium containing amino acids labeled with a heavy isotope. The histone proteins incorporated these heavy amino acids and were dense (**Figure 12.16**). The cells were then transferred to a culture medium that contained amino acids labeled with a light isotope. Histones assembled after the transfer possessed the new, light amino acids and were less dense.



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

12.16 Experimental procedure for studying how nucleosomes dissociate and reassociate in the course of replication.

Description

The illustration shows the question that reads, What happens to histones in eukaryotic DNA replication? The methods below show several flasks used for the following steps. 1, A caption to the first flask with a darker liquid reads, grow cells for several generations in medium that contains amino acids labeled with a heavy isotope. A part of this cell growth is saved and the remaining is transferred to a medium that contains amino acids labeled with a light isotope. 2, A caption referring to a flask of lighter liquid reads, transfer the cells to a medium that contains amino acids labeled with a light isotope. Replication occurs. Octomers from both the flasks are isolated. 3, isolate histone octamers before and after replication. The octomers obtained are spun below the respective flasks. 4, and subject them to density gradient centrifugation. The original cell growth gives a single red

colored band at the bottom of the test tube below, representing old octamers with heavy amino acids. 6, old octamers are dense and will move toward the bottom of the tube. The cell growth after changing medium gives 3 purple colored broad bands at the center of the test tube below, representing octamers with mixture of old and new histones (heavy and light amino acids). 6, newly synthesized octamers are less dense and thus will be higher in the tube. A caption below reads, After D N A replication, the new, reassembled octamers are a

random mixture of old and new histones.

After replication, when the histone octamers were isolated and centrifuged in a density gradient, they formed a continuous band between the positions expected of high-density (old) and low-density (new) octamers. This finding indicates that newly assembled octamers consist of a mixture of old and new histones. Further evidence indicates that reconstituted nucleosomes appear on the new DNA molecules quickly after the new DNA emerges from the replication machinery.

The reassembly of nucleosomes during replication is facilitated by proteins called histone chaperones, which are associated with the helicase enzyme that unwinds the DNA. The histone chaperones accept old histones from the original DNA molecule and deposit them, along with newly synthesized histones, on the two new DNA molecules. Current evidence suggests that the original histone octamer is broken down into two H2A–H2B dimers (each dimer consisting of one H2A and one H2B) and a single H3–H4 tetramer (each tetramer consisting of two H3 histones and two H4 histones). The old H3–H4 tetramer is then transferred randomly to one of the new DNA molecules and serves as a foundation onto which either new or old copies of H2A–H2B dimers are added. Newly synthesized H3–H4 tetramers and H2A–H2B dimers are also added to each new DNA molecule to complete the formation of new nucleosomes. The assembly of the new

nucleosomes is facilitated by a protein called chromatin-assembly factor 1 (CAF-1). ❯ **TRY** PROBLEM 31

CONCEPTS

After DNA replication, new nucleosomes quickly reassemble on the two new molecules of DNA. Nucleosomes break down in the course of replication and reassemble from a mixture of old and new histones. The reassembly of nucleosomes during replication is facilitated by histone chaperones and chromatin-assembly factors.

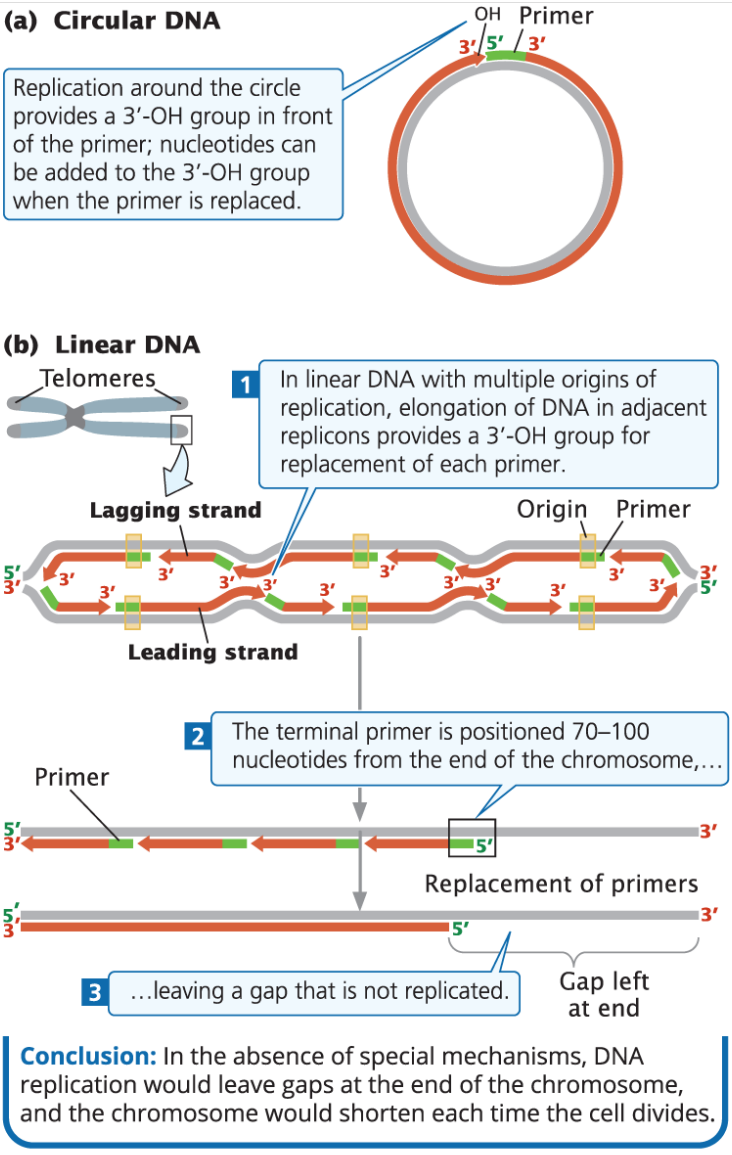
The Location of Replication Within the Nucleus

The DNA polymerases that carry out replication are frequently depicted as moving down the DNA template, much as a locomotive travels along a train track. Evidence suggests that this view is incorrect. A more accurate view is that the polymerase is fixed in location and the template DNA is threaded through it, with newly synthesized DNA molecules emerging from the other end.

Techniques of fluorescence microscopy, which are able to reveal active sites of DNA synthesis, show that most replication in the nucleus of a eukaryotic cell takes place at a limited number of fixed sites, often referred to as replication factories. Time-lapse micrographs reveal that newly duplicated DNA is extruded from these particular sites. Similar results have been obtained for bacterial cells.

Replication at the Ends of Chromosomes

A fundamental difference between eukaryotic and bacterial replication arises because eukaryotic chromosomes are linear and thus have ends. As already stated, the 3′-OH group needed by DNA polymerases is provided at the initiation of replication by RNA primers that are synthesized by primase. This solution is temporary, however, because eventually the primers must be removed and replaced by DNA nucleotides. In a circular DNA molecule, elongation around the circle eventually provides a 3′-OH group immediately in front of the primer (**Figure 12.17a**). After the primer has been removed, the replacement DNA nucleotides can be added to this 3′-OH group. But what happens when a DNA molecule is not circular but linear?



12.17 DNA synthesis at the ends of circular and linear chromosomes must differ.

Description

Illustration a, at the top, shows a circular D N A molecule and at its 3 prime end, an O H group is labeled. A primer is adjacent that runs from 5 prime to 3 prime. A caption reads, replication around the circle provides a 3 prime O H group in front of the primer; nucleotides can be added to the 3 prime O H group when the primer is replaced. Illustration b below shows an X-shaped chromosome with its tips labeled as telomeres. A zoomed out illustration shows three replication bubbles on a stretch of D N A. All the three replication bubbles have origins in the center (one on top strand and one on bottom strand). Continuous leading strands are elongating from left to right for bottom

template strand and right to left for the top template strand. The lagging strands are also elongating from right to left in top strand and left to right in bottom strand. A caption reads, in linear D N A with multiple origins of replication, elongation of D N A in adjacent replicons provides a 3 prime O H group for replacement of each primer. In the next step below, multiple discontinuous strands with markers at 5 prime end are marked from right to left. A caption reads, the terminal primer is positioned 70 to 100 nucleotides from the end of the chromosome. In the next step below, replacement of primers occur and a continuous strand is produced running from 3 prime to 5 prime. A gap is left at the end of this strand. A caption reads, leaving a gap that is not replicated. A caption below reads, Conclusion: In the absence of special mechanisms, D N A replication would leave gaps at the end of the chromosome, and the chromosome would shorten each time the cell divides.

THE END-REPLICATION PROBLEM

In linear chromosomes with multiple origins, the elongation of DNA in adjacent replicons provides a 3′-OH group preceding each primer (Figure 12.17b). At the very end of a linear chromosome, however, there is no adjacent stretch of replicated DNA to provide this crucial 3′-OH group. When the terminal primer at the end of the chromosome has been removed, it cannot be replaced by DNA nucleotides, so its removal produces a gap at the end of the chromosome, suggesting that the chromosome should become progressively shorter with each round of replication. This situation has been termed the end-replication problem.

The end-replication problem, as originally proposed, assumed that the terminal primer is located at the very end of the chromosome. Experimental evidence suggests that in some single-celled eukaryotes, such as yeast and some protozoans, the terminal primer is indeed placed at the very end of the chromosome, but this has not been demonstrated for more complex multicellular eukaryotes. Furthermore, chromosome ends in humans are known to shorten at a much faster rate than would be expected if only the terminal primer (which is only about 10 nucleotides long) was

not replaced. Research has now demonstrated that in replication of human chromosomes, the terminal primer is positioned not at the end of the chromosome but rather some 70 to 100 nucleotides from the end (see Figure 12.17b). This means that 70 to 100 nucleotides of DNA at the end of the chromosome are not replicated during the division of somatic cells, and the chromosome shortens by this amount each time the cell divides.

TELOMERES AND TELOMERASE

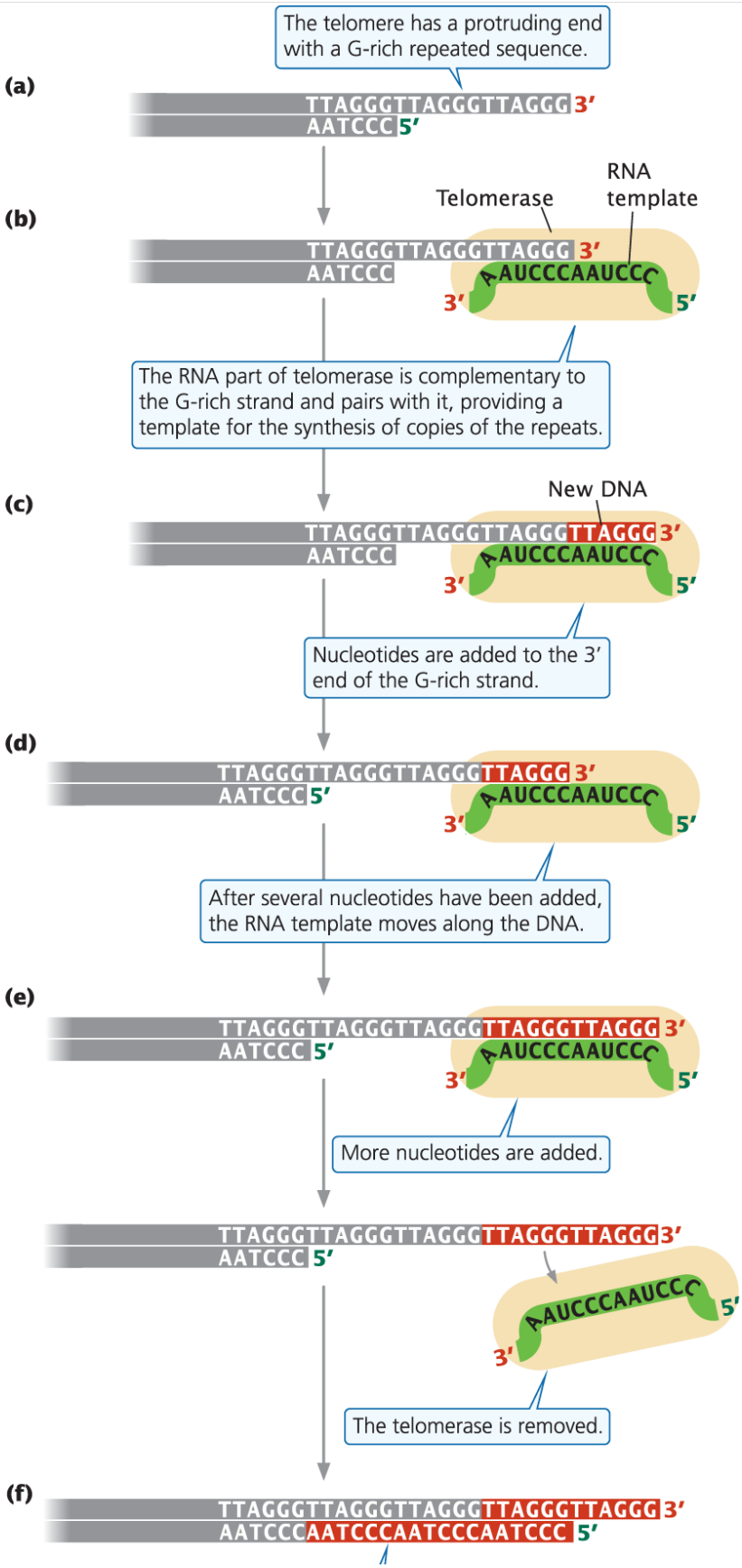
The end-replication problem suggests that chromosomes in eukaryotic cells should shorten with each cell division and eventually self-destruct. In single-celled eukaryotes, germ cells, and early embryonic cells, however, chromosomes do not shorten. So how are the ends of linear chromosomes in these cells replicated?

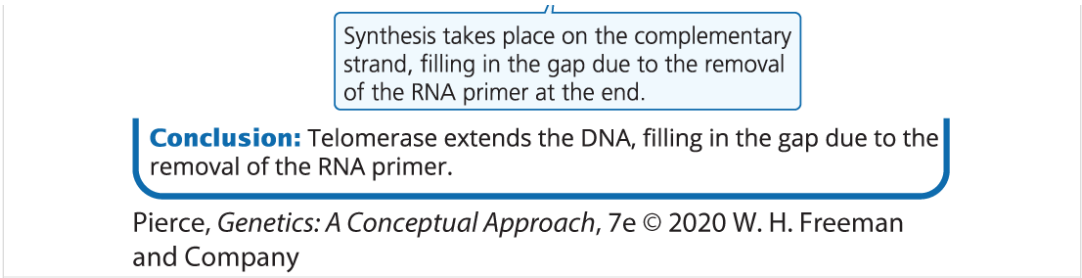
The ends of eukaryotic chromosomes—the telomeres—possess several unique features, one of which is the presence of many copies of a short repeated sequence. In humans, this telomeric repeat is TTAGGG (see Section 11.2). The strand containing this G-rich repeat typically protrudes beyond the complementary C-rich strand (**Figure 12.18a**; see also the section Telomere Structure in Chapter 11):

$$\text{toward centromere} \quad \leftarrow \quad \begin{matrix} 5'\text{-TTAGGGTTAGGGTTAGGG-}3' \\ 3'\text{-AATCCC-}5' \end{matrix} \quad \rightarrow \quad \text{end of ch}$$

The single-stranded protruding end of the telomere, known as the **G-rich 3′ overhang**, can be extended by **telomerase**, an enzyme that has both a protein and an RNA component (also known as a ribonucleoprotein). The RNA component of the enzyme contains from 15 to 22 nucleotides that are

complementary to the sequence on the G-rich strand. This RNA sequence pairs with the G-rich 3′ overhang (**Figure 12.18b**) and provides a template for the synthesis of additional DNA copies of the repeats. DNA nucleotides are added to the 3′ end of the G-rich strand one at a time (**Figure 12.18c**); after several nucleotides have been added, the RNA template moves down the DNA, and more nucleotides are added to the 3′ end of the G-rich strand (**Figure 12.18d**). In this way, the telomerase can extend the 3′ end of the chromosome without the use of a complementary DNA template (**Figure 12.18e**). How the complementary C-rich strand is synthesized (**Figure 12.18f**) is not clear. It may be synthesized by conventional replication, with DNA polymerase α synthesizing an RNA primer on the 5′ end of the extended (G-rich) template. The removal of this primer once again leaves a gap at the 5′ end of the chromosome, but this gap does not matter because the end of the chromosome is extended at each replication by telomerase, so the chromosome does not become shorter overall.


The telomere has a protruding end with a G-rich repeated sequence.
(a)
TTAGGGTTAGGGTTAGGG 3′
AATCCC 5′
(b)
Telomerase
RNA template
TTAGGGTTAGGGTTAGGG 3′
AATCCC
AAUCCCAAUCCC
3′
5′
The RNA part of telomerase is complementary to the G-rich strand and pairs with it, providing a template for the synthesis of copies of the repeats.
(c)
New DNA
TTAGGGTTAGGGTTAGGGTTAGGG 3′
AATCCC
AAUCCCAAUCCC
3′
5′
Nucleotides are added to the 3′ end of the G-rich strand.
(d)
TTAGGGTTAGGGTTAGGGTTAGGG 3′
AATCCC 5′
AAUCCCAAUCCC
3′
5′
After several nucleotides have been added, the RNA template moves along the DNA.
(e)
TTAGGGTTAGGGTTAGGGTTAGGGTTAGGG 3′
AATCCC 5′
AAUCCCAAUCCC
3′
5′
More nucleotides are added.
TTAGGGTTAGGGTTAGGGTTAGGGTTAGGG 3′
AATCCC 5′
AAUCCCAAUCCC
3′
5′
The telomerase is removed.
(f)
TTAGGGTTAGGGTTAGGGTTAGGGTTAGGG 3′
AATCCCAATCCCAATCCCAATCCC 5′



12.18 The enzyme telomerase is responsible for the replication of chromosome ends.

Description

The illustration shows telomere lengthening with 7 parts labeled “a” to “f”. Part “a” at the top shows the G-rich repeated sequence at the end of telomere. A caption reads, the telomere has a protruding end with a G-rich repeated sequence. Part “b” below shows telomerase with its complementary R N A template attaching to the telomere and providing an overhanging template strand for further D N A elongation. A caption reads, the R N A part of telomerase is complementary to the G-rich strand and pairs with it, providing a template for the synthesis of copies of the repeats. Part “c” below shows new D N A added, making the single-stranded section of the D N A longer. A caption reads, Nucleotides are added to the 3 prime end of the G-rich strand. Part “d” below shows the R N A template moving along the newly synthesized D N A nucleotides. A caption reads, after several nucleotides have been added, the R N A template moves along the D N A. Part “e” below shows more nucleotides added to the D N A strand thereby making the strand even longer. A caption reads, more nucleotides are added. This leads to the next part where the telomerase dissociates. A caption reads, the telomerase is removed. Part “f” below shows synthesis occurring on the complementary strand eventually filling in the gap. A caption reads, synthesis takes place on the complementary strand, filling in the gap due to the removal of the R N A primer at the end. Telomerase extends the length of the D N A and fills in the gap at the ends that would have been left by the R N A primer. A text below reads, Conclusion: Telomerase extends the D N A, filling in the gap due to the removal of the R N A primer.

Telomerase is present in single-celled eukaryotes, germ cells, early embryonic cells, and certain proliferative somatic cells (such as bone-marrow cells and cells lining the intestine), all of which must undergo continuous cell division. Most somatic cells have little or no telomerase activity, and chromosomes in these cells progressively shorten with each cell division. These cells are capable of only a limited number of divisions; when the telomeres have shortened beyond a critical point, the

chromosomes become unstable, have a tendency to undergo rearrangements, and are degraded. These events lead to cell death.

THINK-PAIR-SHARE Question 6

CONCEPTS

The ends of eukaryotic chromosomes are replicated by a ribonucleoprotein called telomerase. This enzyme adds extra nucleotides to the G-rich DNA strand of the telomere.

✔ CONCEPT CHECK 10

What would be the result if an organism's telomerase were mutated and nonfunctional?

a. No DNA replication would take place.
b. The DNA polymerase enzyme would stall at the telomere.
c. Chromosomes would shorten with each new generation.
d. RNA primers could not be removed.

TELOMERASE, AGING, AND DISEASE

The shortening of telomeres may contribute to the process of aging. The telomeres of genetically engineered mice that lack a functional telomerase gene (and therefore do not express telomerase in either somatic or germ cells) undergo progressive shortening in successive generations. After several generations, these mice show some signs of premature aging, such as graying, hair loss, and delayed wound healing. Through genetic engineering, it is also possible to create somatic cells that express telomerase. In these cells, telomeres do not shorten, cell aging is inhibited, and the cells divide indefinitely.

Some of the strongest evidence that telomere length is related to aging comes from studies of telomeres in birds. In 2012, scientists in the United Kingdom measured telomere length in red blood cells taken from 99 zebra finches at various times during their lives. The scientists found a strong correlation between telomere length and longevity: birds with longer telomeres lived longer than birds with short telomeres. The strongest predictor of life span was telomere length early in life, at 25 days, which is roughly equivalent to human adolescence. Although these observations suggest that telomere length is associated with aging in some animals, the precise role of telomeres in *human* aging remains uncertain.

Some diseases are associated with abnormalities of telomere replication. People who have Werner syndrome, an autosomal recessive disease, show signs of premature aging that begin in adolescence or early adulthood, including wrinkled skin, graying of the hair, baldness, cataracts, and muscle atrophy. They often develop cancer, osteoporosis, heart and artery disease, and other ailments typically associated with aging. The causative gene, *WRN*, has been mapped to human chromosome 8 and normally encodes a RecQ helicase enzyme, which is necessary for the efficient replication of telomeres. In people who have Werner syndrome, this enzyme is defective; consequently, the telomeres shorten prematurely.

Another disease associated with abnormal maintenance of telomeres is dyskeratosis congenita (DKC), which leads to progressive bone-marrow failure, in which the bone marrow does not produce enough new blood cells. People with an X-linked form of the disease have a mutation in a gene that encodes dyskerin, a protein that normally helps process the RNA component of telomerase. People who have the disease typically inherit short telomeres from a parent who carries the mutation and who is unable

to maintain telomere length in his or her germ cells owing to defective dyskerin. In families that carry this mutation, telomere length typically shortens with each successive generation, leading to anticipation: a progressive increase in the severity of the disease over generations (see Section 5.4).

Telomerase also appears to play a role in cancer. Cancer cells have the capacity to divide indefinitely, and telomerase is expressed in 90% of all cancers. Some recent evidence indicates that telomerase may stimulate cell proliferation independently of its effect on telomere length, so the mechanism by which telomerase contributes to cancer is not clear. As we will see in Chapter 23, cancer is a complex, multistep process that usually requires mutations in at least several genes. Telomerase activation alone does not lead to cancerous growth in most cells, but it does appear to be required, along with other mutations, for cancer to develop. Some experimental anticancer drugs work by inhibiting the action of telomerase.

One of the difficulties in studying the effect of telomere shortening on the aging process is that the expression of telomerase in somatic cells also promotes cancer, which may shorten a person's life span. To circumvent this problem, Antonia Tomás-Loba and her colleagues created genetically engineered mice that expressed telomerase and carried genes that made them resistant to cancer. These mice had longer telomeres, lived longer, and exhibited fewer age-related changes, such as skin alterations, decreases in neuromuscular coordination, and degenerative diseases, than did normal mice. These results support the idea that telomere shortening contributes to aging. › **TRY PROBLEM 33**

THINK-PAIR-SHARE Question 7

Replication in Archaea

The process of replication in archaea has a number of features in common with replication in eukaryotic cells. Many of the proteins taking part are more similar to those in eukaryotic cells than to those in bacteria. Like bacteria, some archaea have a single origin of replication, but many archaea have multiple origins, as eukaryotes do (although archaea have far fewer origins than are found in most eukaryotic chromosomes). The origins of archaea do not contain the typical sequences recognized by bacterial initiator proteins; instead, they have sequences that are similar to those found in some eukaryotic origins. The initiator proteins of archaea are also more similar to those of eukaryotes than to those of bacteria. These similarities in replication between archaeal and eukaryotic cells reinforce the conclusion that the archaea are more closely related to eukaryotic cells than to the prokaryotic bacteria.

12.5 Recombination Takes Place Through the Alignment, Breakage, and Repair of DNA Strands

Recombination is the exchange of genetic information between DNA molecules; when the exchange is between homologous DNA molecules, it is called **homologous recombination**. This process takes place in crossing over, in which homologous regions of chromosomes are exchanged and alleles are shuffled into new combinations (see Figure 7.5). Recombination is an extremely important genetic process because it increases genetic variation. Rates of recombination provide important information about linkage relations among genes, which is used to create genetic maps (see Figures 7.13 and 7.14). Recombination is also essential for some types of DNA repair (as we will see in Chapter 18).

Homologous recombination is a remarkable process: a nucleotide strand of one chromosome aligns precisely with a nucleotide strand of the homologous chromosome, breaks arise in corresponding regions of the two DNA molecules, parts of the molecules precisely change place, and then the pieces are correctly joined. In this complicated series of events, no genetic information is lost or gained. Although the precise molecular mechanism of homologous recombination is still not completely understood, the exchange is probably accomplished through the pairing of complementary

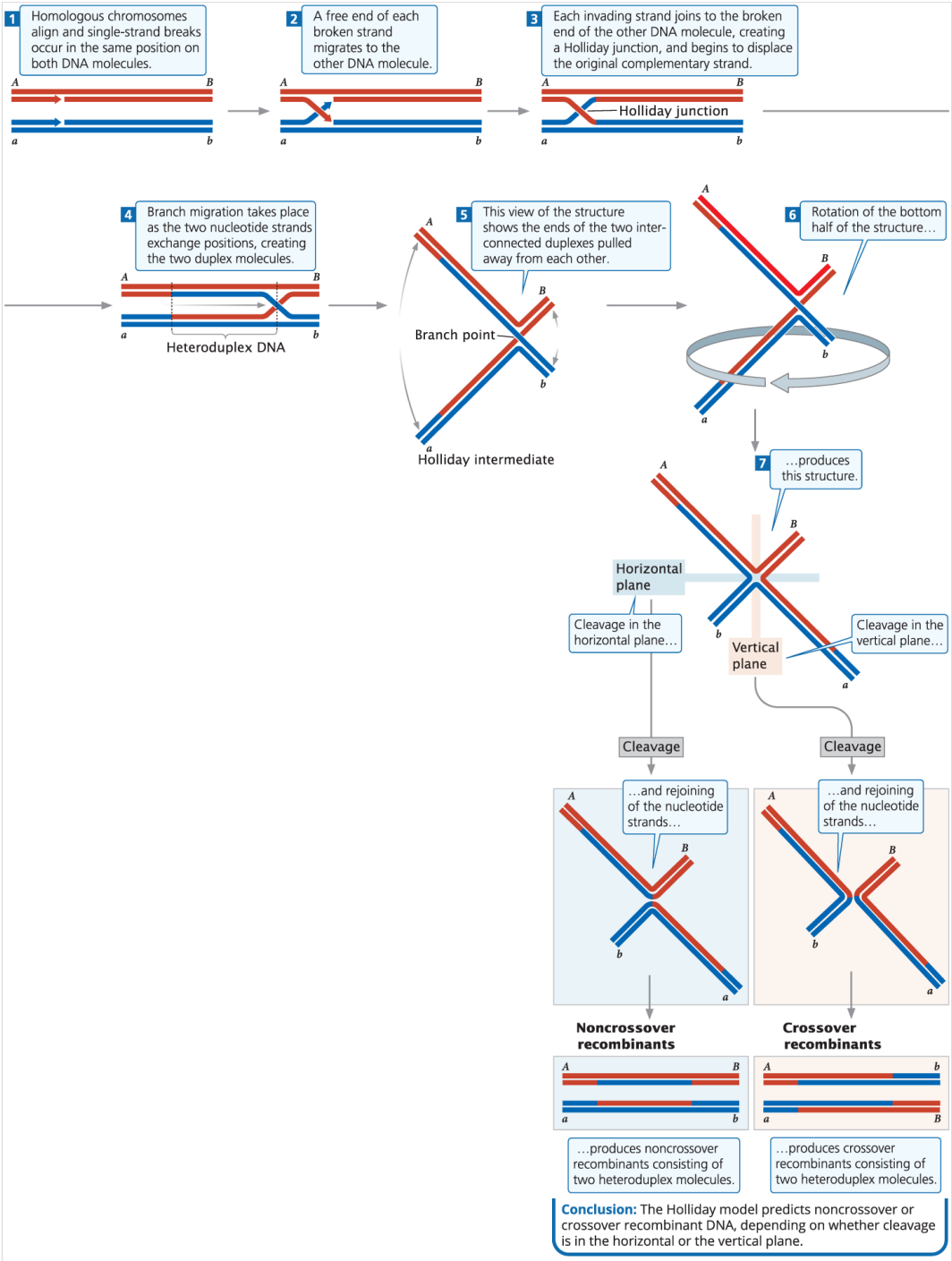
bases. A single nucleotide strand of one chromosome pairs with the complementary strand of another, forming **heteroduplex DNA**, which is DNA consisting of nucleotide strands from different sources (see the next section and Figure 12.19).

In meiosis, homologous recombination (crossing over) could theoretically take place before, during, or after DNA synthesis. Cytological, biochemical, and genetic evidence indicates that it takes place in prophase I of meiosis, whereas DNA replication takes place earlier, in interphase. Thus, crossing over must entail the breaking and rejoining of chromatids when homologous chromosomes are at the four-strand stage (see Figure 7.5). This section explores some theories about how the process of recombination takes place.

Models of Recombination

Homologous recombination takes place through several different pathways. One pathway is initiated by a single-strand break in each of two DNA molecules and includes the formation of a special structure called the **Holliday junction** (**Figure 12.19**). In this model, the double-stranded DNA molecules of two homologous chromosomes align precisely. A single-strand break in each of the DNA molecules provides a free end that invades and joins the free end of the other DNA molecule. Thus, strand invasion and joining take place on both DNA molecules, creating two heteroduplex DNAs, each consisting of one original strand plus one new strand from the

other DNA molecule. The point at which the nucleotide strands pass from one DNA molecule to the other is the Holliday junction. The junction moves along the molecules in a process called branch migration.



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12.19 The Holliday model of homologous recombination. In this model, recombination takes place through single-strand breaks, strand displacement, branch migration, and resolution of a single Holliday junction.

Description

The illustration shows 2 pairs of homologous chromosomes, represented by lines, on the right. There are red chromosomes with genes big A and big B and blue chromosomes with genes small A and small B. The bottom red chromosome and the upper blue strand have a nick. A caption reads, homologous chromosomes align and single-strand breaks occur in the same position on both D N A molecules. In the next step on the right, a free end from each of the broken strand migrates towards the other D N A molecule. Text reads, a free end of each broken strand migrates to the other D N A molecule. In the next step on the right, the broken strand joins the remaining part of the other D N A molecule and the junction is known as holiday junction. A caption reads, each invading strand joins to the broken end of the other D N A molecule, creating a Holliday junction, and begins to displace the original complementary strand.

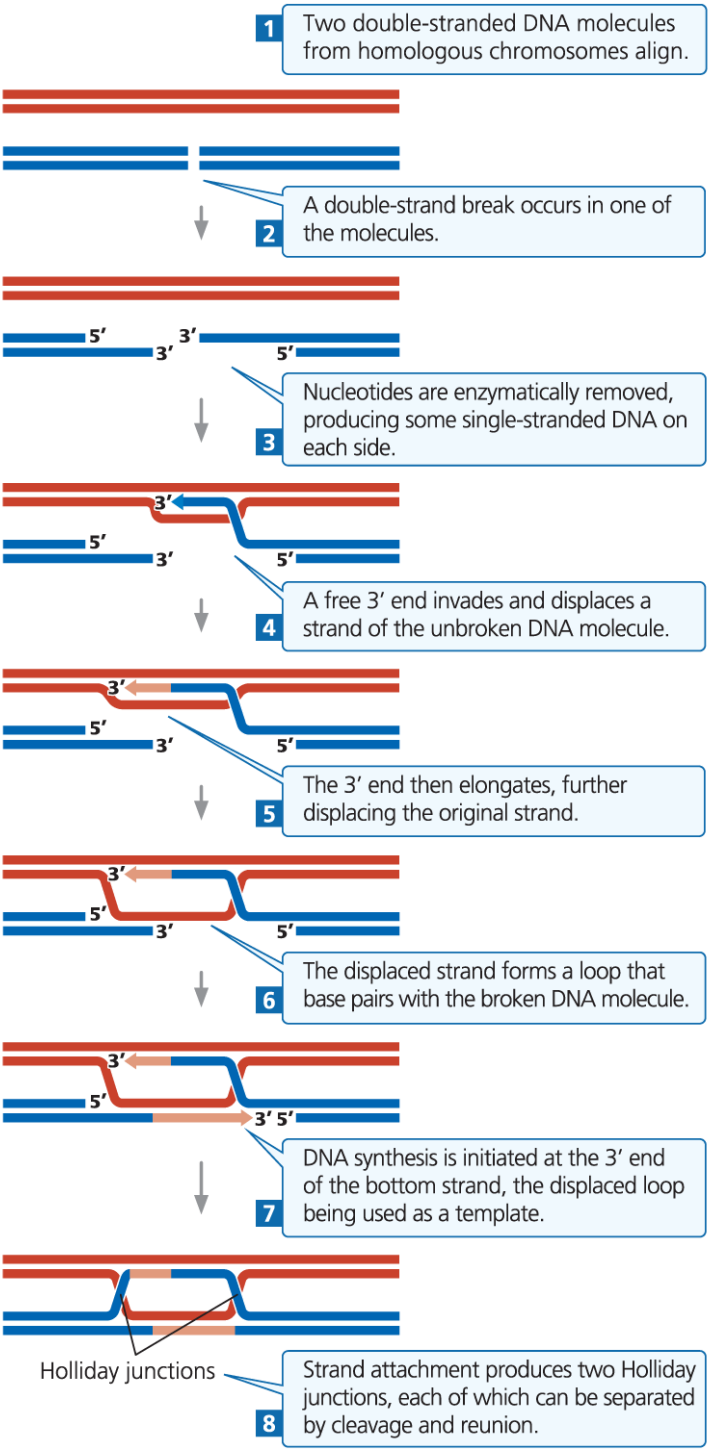
The illustration shows different alignments of the heteroduplex, beginning with strands invading and the creation of a heteroduplex D N A, or the areas where the blue and red D N A strands, represented by lines, are complexed together. A caption reads, branch migration takes place as the two nucleotide strands exchange positions, creating the two duplex molecules. In the next step on the right, the ends of the interconnected duplexes are then pulled away from each other while they still are joined at the branch point. A caption reads, This view of the structure shows the ends of the 2 interconnected duplexes pulled away from one another. In the next step on the right, the bottom half rotates in a clockwise direction. A caption reads, rotation of the bottom half of the structure. In the next step, D N A strands align in the shape of a cross with each arm at an angle of 90 degrees from the other. The alignment is diagonal to the horizontal plane and vertical plane such that the long arms are at 45 degree angle from both horizontal and vertical plane. The long arms of the cross are heteroduplexed D N A, and the shorter arms are one red and one blue. A caption reads, produces this structure. Cleavage in horizontal plane leads to a chromosome below with genes big A and big B and other with small A and small B, even though the middle portion of the chromosome between the 2 genes are hybrid in color, red and blue. This further leads to formation of noncrossover recombinants below consisting of 2 heteroduplex molecules (one is red with genes big A big B with a portion of blue in middle of the lower chromosome; other is blue with genes small A small B and red portion in the middle of upper chromosome). A caption reads, cleavage in the horizontal plane and rejoining of the nucleotide strands produces noncrossover recombinants consisting of two heteroduplex molecules. Cleavage in the vertical plane leads to chromosome below with genes with big A small B on one side, and the other chromosome with genes small A big

B. This further leads to crossover recombinants below consisting of 2 heteroduplex molecules (one is hybrid with genes big A small B; other is hybrid with genes small A big B). A caption reads, cleavage in the vertical plane and rejoining of the nucleotide strands produces crossover recombinants consisting of two heteroduplex molecules.

The exchange of nucleotide strands and branch migration produce a structure termed the Holliday intermediate, which can be cleaved in one of two ways. Cleavage in the horizontal plane, followed by rejoining of the strands, produces noncrossover recombinants, in which the genes on the two ends of the molecules are identical with those originally present (gene *A* with gene *B*, and gene *a* with gene *b*). Cleavage in the vertical plane, followed by rejoining, produces crossover recombinants, in which the genes on the two ends of the molecules are different from those originally present (gene *A* with gene *b*, and gene *a* with gene *B*).

Another pathway for recombination is initiated by double-strand breaks in one of the two aligned DNA molecules (**Figure 12.20**). In this model, the removal of some nucleotides at the ends of the broken strands—followed by strand invasion, displacement, and replication—produces two heteroduplex DNA molecules joined by two Holliday junctions. The interconnected molecules produced in the double-strand-break model can be separated by further cleavage and reunion of the nucleotide strands in the same way that the Holliday intermediate is separated in the single-strand-break model. Whether crossover or noncrossover molecules are produced depends on whether cleavage is in the vertical or the horizontal

plane. View (A) **Animation 12.5** to see how the Holliday and double-strand-break models lead to recombination.



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12.20 The double-strand-break model of recombination. In this model, recombination takes place through a double-strand break in one DNA duplex, strand displacement, DNA synthesis, and the resolution of two Holliday junctions.

Description

The illustration shows two double stranded D N A molecules that are represented by lines. The chromsomes are present at the top, where one is red and other is blue. A double strand break occurs in the blue D N A molecule present below. A caption reads, two double-stranded D N A molecules from homologous chromosomes align. A double-strand break occurs in one of the molecules. Few nucleotides around the break are enzymatically removed in the next step below. The top blue strand has 5 prime end and the remaining part begins with 3 prime end. The bottom blue strand has 3 prime end and the remaining part begins with 5 prime end. A caption reads, nucleotides are enzymatically removed, producing some single-stranded D N A on each side. In the next step below, the 3 prime end of the top blue strand invades and displaces the lower red D N A strand. A caption reads, a free 3 prime end of the broken blue strand invades and displaces a strand of the unbroken D N A molecule. In the next step below, the 3 prime end of the blue strand elongates and keeps on displacing the red strand. A caption reads, the 3 prime end then elongates, further displacing the original strand. In the next step below, the displaced D N A forms a loop and base pairs with the broken blue D N A strand. A caption reads, the displaced strand forms a loop that base pairs with the other strand of the broken D N A molecule. In the next step below, D N A synthesis begins at the 3 prime end of the bottom blue strand with the displaced red strand as a template. A caption reads, D N A synthesis is initiated at the 3 prime end of the bottom strand, the displaced loop being used as a template. Finally in the last step below, the 5 prime end of the top blue strand extends upwards and joins to the elongated 3 prime end of the same blue strand and 2 Holliday junctions are produced. A caption reads, Strand attachment produces 2 Holliday junctions, each of which can be separated by cleavage and reunion.

Evidence for the double-strand-break model originally came from results of genetic crosses in yeast that could not be explained by the Holliday model. Subsequent observations showed that double-strand breaks appear in yeast in prophase I, when crossing over takes

place, and that mutant strains that are unable to form double-strand breaks do not exhibit meiotic recombination. Although considerable evidence supports the double-strand-break model in yeast, the extent to which it applies to other organisms is not yet known.

CONCEPTS

Homologous recombination requires the formation of heteroduplex DNA consisting of one nucleotide strand from each of two homologous chromosomes. In the Holliday model, homologous recombination is accomplished through a single-strand break in each DNA molecule, strand displacement, and branch migration. In the double-strand-break model, recombination is accomplished through double-strand breaks, strand displacement, and branch migration.

✔ CONCEPT CHECK 11

Why is recombination important?

Enzymes Required for Recombination

Recombination between DNA molecules requires the unwinding of DNA helices, the cleavage of nucleotide strands, strand invasion, and branch migration, followed by further strand cleavage and union to remove Holliday junctions. The molecular mechanism of recombination has been extensively studied in *E. coli*. Although bacteria do not undergo meiosis, they do have a type of sexual reproduction (conjugation), in which one bacterium donates its

chromosome to another (discussed more fully in Section 9.3). Subsequent to conjugation, the recipient bacterium has two chromosomes, which may undergo homologous recombination. Geneticists have isolated mutant strains of *E. coli* that are deficient in recombination; the study of these strains has resulted in the identification of genes and proteins that take part in bacterial recombination, revealing several different pathways by which it can take place.

Three genes that play pivotal roles in *E. coli* recombination are *recB*, *recC*, and *recD*, which encode three polypeptides that together form the RecBCD protein. This protein unwinds double-stranded DNA and is capable of cleaving nucleotide strands. The *recA* gene encodes the RecA protein; this protein allows invasion of a DNA helix by a single strand and the subsequent displacement of one of the original strands. In eukaryotes, the formation and branch migration of Holliday structures is facilitated by the enzyme Rad51.

In *E. coli*, *ruvA* and *ruvB* genes encode proteins that catalyze branch migration, and the *ruvC* gene produces a protein, called resolvase, that cleaves Holliday structures. Cleavage and resolution of Holliday structures in eukaryotes is carried out by an analogous enzyme called GEN1. Single-strand-binding proteins, DNA ligase, DNA polymerases, and DNA gyrase also play roles in various types of recombination, in addition to their functions in DNA replication.

CONCEPTS

A number of proteins have roles in recombination, including RecA, RecBCD, RuvA, RuvB, resolvase, single-strand-binding proteins, DNA ligase, DNA polymerases, and DNA gyrase.

✔ CONCEPT CHECK 12

What is the function of resolvase in recombination?

a. It unwinds double-stranded DNA.
b. It allows a single DNA strand to invade a DNA helix.
c. It displaces one of the original DNA strands during branch migration.
d. It cleaves the Holliday structure.

Chapter 12 Review

CHAPTER SUMMARY

Section 12.2 Learning Objective: Be able to describe the process of semiconservative replication.

- DNA replication is semiconservative: The two nucleotide strands of DNA separate, and each serves as a template on which a new strand is synthesized.
- In theta replication, the two nucleotide strands of a circular DNA molecule unwind, creating a replication bubble. Within each replication bubble, DNA is normally synthesized on both strands and at the replication fork, producing two circular DNA molecules.
- Rolling-circle replication is initiated by a break in one strand of circular DNA, which produces a 3′-OH group to which new nucleotides are added while the 5′ end of the broken strand is displaced from the circle.
- Linear eukaryotic DNA contains many origins of replication. Unwinding and replication take place on both templates at both ends of the replication bubble until adjacent replicons meet, resulting in two linear DNA molecules.
- All DNA synthesis is in the 5′ → 3′ direction. Because the two nucleotide strands of DNA are antiparallel, replication takes

place continuously on one strand (the leading strand) and discontinuously on the other (the lagging strand).

Section 12.3 Learning Objective: Be able to outline the mechanism of bacterial replication.

- Replication in bacteria begins when an initiator protein binds to an origin of replication and unwinds a short stretch of DNA, to which DNA helicase attaches. DNA helicase unwinds the DNA at the replication fork, single-strand-binding proteins bind to the single nucleotide strands to prevent secondary structures, and DNA gyrase (a topoisomerase) removes the strain ahead of the replication fork that is generated by unwinding.
- During replication, primase synthesizes short primers consisting of RNA nucleotides, providing a 3′-OH group to which DNA polymerase can add DNA nucleotides.
- DNA polymerase adds new nucleotides to the 3′ end of a growing polynucleotide strand. Bacteria have two DNA polymerases that have primary roles in replication: DNA polymerase III, which synthesizes new DNA on the leading and lagging strands, and DNA polymerase I, which removes and replaces primers.
- DNA ligase seals the nicks that remain in the sugar–phosphate backbone when the RNA primers are replaced by DNA nucleotides.
- Several mechanisms ensure the high rate of accuracy in replication, including precise nucleotide selection, proofreading, and mismatch repair.

Section 12.4 Learning Objective: Be able to summarize the differences in eukaryotic and bacterial replication.

- Precise replication at multiple origins in eukaryotes is ensured by licensing factors that must attach to an origin before replication can begin.
- Eukaryotic nucleosomes are quickly assembled on new molecules of DNA; newly assembled nucleosomes consist of a random mixture of old and new histone proteins.
- The ends of linear eukaryotic DNA molecules are replicated by the enzyme telomerase. The shortening of telomeres in somatic cells may contribute to aging.
- The process of replication in archaea has some features in common with replication in eukaryotes.

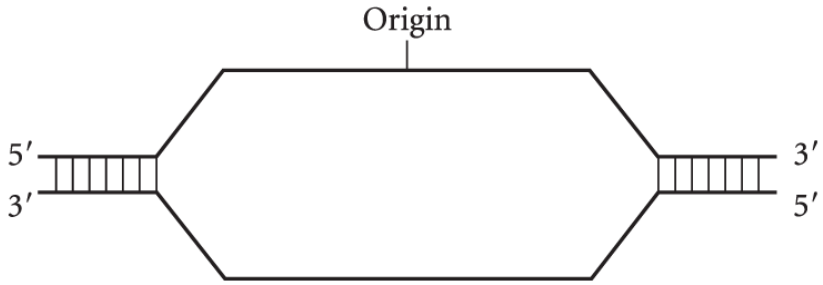
Section 12.5 Learning Objective: Be able to explain the molecular mechanism of homologous recombination.

- Homologous recombination takes place through alignment of homologous DNA segments, breaks in nucleotide strands, and rejoining of the strands. Homologous recombination requires a number of enzymes and proteins.

WORKED PROBLEMS

Problem 1

The following diagram represents the template strands of a replication bubble in a DNA molecule. Draw in the newly synthesized strands, and identify the leading and lagging strands.



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Description
The top strand has 5 prime end on the left side and the bottom strand has 5 prime end on the right side. In the middle a replication bubble is formed with origin labeled in the middle.

» Solution Strategy

What information is required in your answer to the problem?

The provided diagram with the newly synthesized strands drawn in and the leading and lagging strands labeled.

What information is provided to solve the problem?

A diagram of the template DNA with 5′ and 3′ ends labeled.

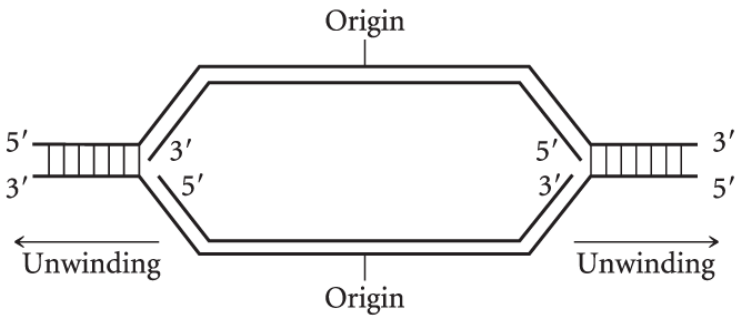
For help with this problem, review:

Direction of Replication in Section 12.2 and Figure 12.10c.

» Solution Steps

To determine the leading and lagging strands, first note which end of each template strand is 5′ and which end is 3′. With a pencil, draw in the strands being synthesized on these templates, and identify their 5′ and 3′ ends.

Recall: The two strands of DNA are antiparallel, so the newly synthesized strands should have a polarity opposite to the template strand.



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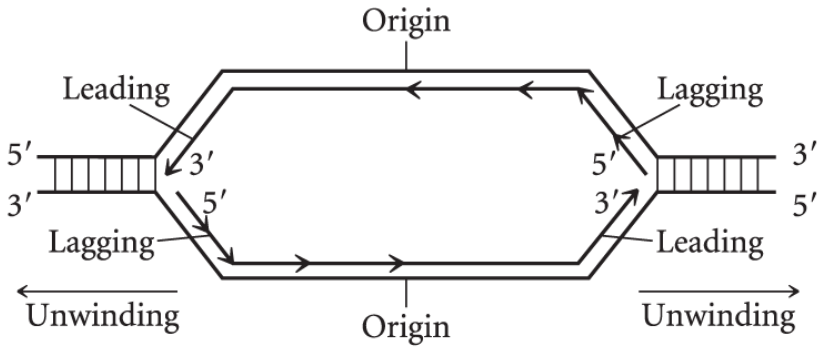
Description
The top strand has 5 prime end on the left side, and the bottom strand has 5 prime end on the right side. In the middle, a replication bubble is present with the origin labeled in the center. Within the bubble, below the top strand a new strand has 3 prime end on the left and 5 prime end on the right. Above the bottom strand a new strand has 5 prime end on the left and 3 prime on the right. Below both double-stranded D N A segments, arrows are extending outwards and labeled unwinding. A caption reads, recall the 2 strands of D N A are antiparallel, so the newly synthesized strand should have the opposite polarity form the template strand.

Next, determine the direction of replication for each new strand, which must be 5′ → 3′. You might draw arrows on the new strands to indicate the direction of replication. After you have established the direction of replication for each strand, look at each replication fork and determine whether the direction of replication for a strand is the same as the direction of unwinding. The strand on which replication is in the same direction as that of unwinding is the leading strand. The strand on which replication is in the direction opposite that of unwinding is the lagging strand.

Recall: DNA synthesis is always 5′ to 3′.



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Description
The line drawing shows double stranded D N A with a replication bubble in between. The replication bubble has the newly synthesized strands within. Below the top strand, a newly synthesized strand is present with its top left end (3 prime) labeled leading strand and the top right end (5 prime) labeled lagging strand. Above the bottom strand, a newly synthesized strand has bottom left (5 prime) labeled lagging strand and the bottom right (3 prime) labeled as leading strand. Two arrows below the double stranded D N A point outward from the center and are labeled unwinding. A caption reads, hint: each replication fork should have one leading and one lagging strand.

Hint: Each replication fork should have one leading and one lagging strand.

Problem 2

Consider the experiment conducted by Meselson and Stahl in which they used ^{14}N and ^{15}N in cultures of *E. coli* and equilibrium density gradient centrifugation. Draw pictures to represent the bands produced by bacterial DNA in the centrifuge tube before the switch to a medium containing ^{14}N and after one, two, and three rounds of replication in that medium. Use separate sets of drawings to show the bands that would appear if replication were (a) semiconservative, (b) conservative, (c) dispersive.

» Solution Strategy

What information is required in your answer to the problem?

Drawings that represent the bands produced by bacterial DNA in the centrifuge tube before the switch to a medium containing ^{14}N and after one, two, and three rounds of replication in that medium; thus, you should have drawings of four tubes for each model of replication. You will need separate sets of drawings for semiconservative, conservative, and dispersive replication.

What information is provided to solve the problem?

- The bacterial DNA was originally labeled with ^{15}N, and then the bacteria were switched to a medium with ^{14}N (see discussion of experiment on pp. 347-348).
- Original DNA will contain ^{15}N. Newly synthesized DNA will contain ^{14}N.
- Equilibrium density gradient centrifugation was performed before the bacteria were switched to ^{14}N and after one, two, and three rounds of replication following the switch.

For help with this problem, review:

Meselson and Stahl's Experiment in Section 12.2.

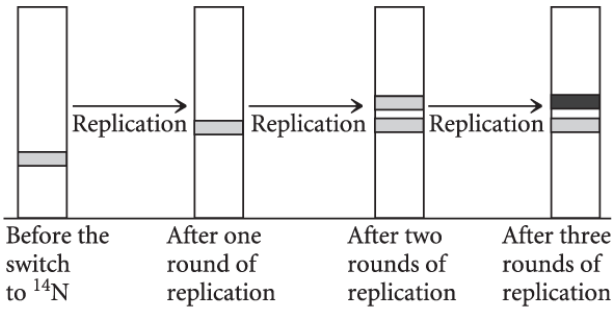
» Solution Steps

DNA labeled with ^{15}N will be denser than DNA labeled with ^{14}N; therefore, ^{15}N-labeled DNA will sink lower in the centrifuge tube. Before the switch to a medium containing ^{14}N, all DNA in the bacteria will contain ^{15}N and will produce a single band in the lower end of the tube.

a. With semiconservative replication, the two strands separate, and each serves as a template on which a new strand is synthesized. After one round of replication, the original template strand of each molecule will contain ^{15}N and the new strand of each molecule will contain ^{14}N, so a single band will appear in the centrifuge tube halfway between the positions expected of DNA containing only ^{15}N and of DNA containing

only ^{14}N. In the next round of replication, the two strands again separate and serve as templates for new strands. Each of the new strands contains only ^{14}N; thus, some DNA molecules will contain one strand with the original ^{15}N and one strand with new ^{14}N, whereas the other molecules will contain two strands with ^{14}N. This labeling will produce two bands, one at the intermediate position and one at a higher position in the tube. Additional rounds of replication should produce increasing amounts of DNA that contains only ^{14}N; so the higher band will get darker.

Hint: Review the distribution of new and old DNA in semiconservative, conservative, and dispersive replication in Figure 12.1.



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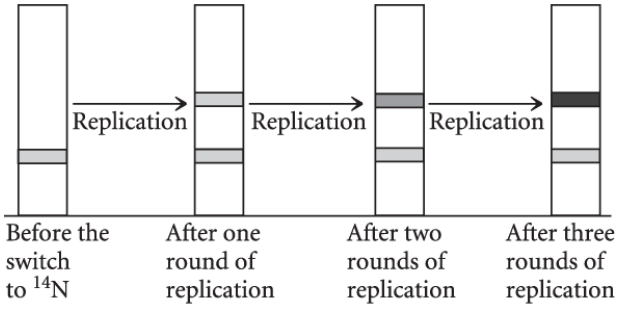
Description

On the left side, a grey band appears near the bottom representing D N A before the switch to N left superscript 14. Replication occurs and a grey band appears just below the center after one round of replication, shown on the right. Replication occurs and two gray bands appear at the center after two rounds of replication, shown on the right. Replication occurs and the above band becomes black while the grey band remains as it is after three rounds of replication shown, on the right.

b. With conservative replication, the entire molecule serves as a template. After one round of replication, some molecules would consist entirely of ^{15}N, and others would consist entirely of ^{14}N; therefore, two bands would be present. Subsequent rounds of replication would increase the fraction of DNA consisting entirely of new ^{14}N; thus, the upper band would get darker. However, the original DNA with ^{15}N would remain, and so two bands would still be present.



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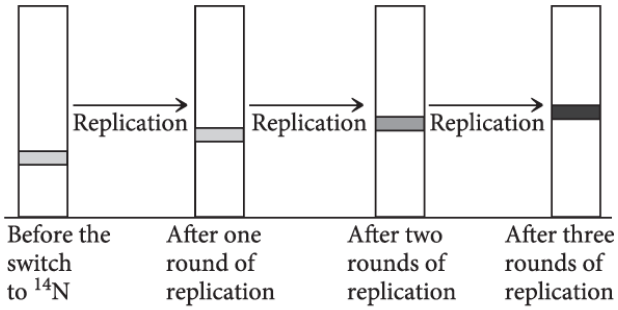
Description

On the left side, a grey band appears near the bottom representing D N A before the switch to N left superscript 14. Replication occurs and two grey bands appear (one at the center and one at the bottom) after one round of replication, shown on the right. Replication occurs and two gray bands appear (one darker grey and at the center and other at the bottom) after two rounds of replication, shown on the right. Replication occurs and the above band becomes black while the grey band remains as it is after three rounds of replication, shown on the right.

c. In dispersive replication, both nucleotide strands break down into fragments that serve as templates for the synthesis of new DNA. The fragments then reassemble into DNA molecules. After one round of replication, all DNA would contain approximately half ^{15}N and half ^{14}N, producing a single band halfway between

the positions expected of DNA labeled with ^{15}N and of DNA labeled with ^{14}N. With further rounds of replication, the proportion of ^{14}N in each molecule would increase; therefore, a single hybrid band would remain, but its position in the tube would move upward. The band would also get darker as the total amount of DNA increased.



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Description

On the left side, a grey band appears near the bottom representing D N A before the switch to N left superscript 14. Replication occurs and one grey bands appears a little below the center after one round of replication, shown on the right. Replication occurs and a dark gray band appears just below the center after two rounds of replication, shown on the right. Replication occurs and a black band appears at the after three rounds of replication, shown on the right.

IMPORTANT TERMS

semiconservative replication
equilibrium density gradient centrifugation
replicon

origin of replication
theta replication
replication bubble
replication fork
bidirectional replication
rolling-circle replication
DNA polymerase
continuous replication
leading strand
discontinuous replication
lagging strand
Okazaki fragment
initiator protein
DNA helicase
single-strand-binding protein (SSB)
DNA gyrase
primase
primer
DNA polymerase III
β sliding clamp
DNA polymerase I
DNA ligase
proofreading
mismatch repair
autonomously replicating sequence (ARS)
origin-recognition complex (ORC)
replication licensing factor
DNA polymerase α
DNA polymerase δ

DNA polymerase ε
translesion DNA polymerase
G-rich 3′ overhang
telomerase
homologous recombination
heteroduplex DNA
Holliday junction

ANSWERS TO CONCEPT CHECKS

1. Two bands
2. b
3. c
4. Initiator protein, helicase, single-strand-binding protein, DNA gyrase.
5. c
6. b
7. c
8. Some differences are the size of eukaryotic genomes, the linear structure of eukaryotic chromosomes, and the association of DNA with histone proteins.
9. Error-prone DNA polymerases can bypass lesions in the DNA helix that stall accurate, high-speed DNA polymerases.
10. c
11. Recombination is important for genetic variation and for some types of DNA repair.
12. d

Chapter 12 Assessment

COMPREHENSION QUESTIONS

Section 12.2

1. What is semiconservative replication?

2. How did Meselson and Stahl demonstrate that replication in *E. coli* takes place in a semiconservative manner?

3. Draw a molecule of DNA undergoing theta replication. On your drawing, identify (a) origin of replication, (b) polarity (5′ and 3′ ends) of all template strands and newly synthesized strands, (c) leading and lagging strands, (d) Okazaki fragments, and (e) locations of primers.

4. Draw a molecule of DNA undergoing rolling-circle replication. On your drawing, identify the (a) origin, (b) template and newly synthesized strands, and (c) 5′ and 3′ ends of template and newly synthesized strands.

5. Draw a molecule of DNA undergoing linear eukaryotic replication. On your drawing, identify (a) origin of replication, (b) polarity (5′ and 3′ ends) of all template and newly

synthesized strands, (c) leading and lagging strands, (d) Okazaki fragments, and (e) locations of primers.

6. What are the three major requirements of replication?

7. What substrates are used in DNA synthesis?

Section 12.3

8. List the different proteins and enzymes taking part in bacterial replication. Give the function of each in the replication process.

9. Why is DNA gyrase necessary for replication?

10. What similarities and differences exist in the enzymatic activities of DNA polymerases I and III? What is the function of each DNA polymerase in bacterial cells?

11. Why is primase required for replication?

12. What three mechanisms ensure the accuracy of replication in bacteria?

Section 12.4

13. How does replication licensing ensure that DNA is replicated only once at each origin per eukaryotic cell cycle?

14. In what ways is eukaryotic replication similar to bacterial replication, and in what ways is it different?

15. What is the end-replication problem? Why, in the absence of telomerase, do the ends of linear chromosomes get progressively shorter each time the DNA is replicated?

16. Outline in words and pictures how telomeres at the ends of eukaryotic chromosomes are replicated.

Section 12.5

17. Explain how the type of cleavage of the Holliday intermediate leads to noncrossover recombinants and crossover recombinants.

18. What are some of the enzymes taking part in recombination in *E. coli*, and what roles do they play?

APPLICATION QUESTIONS AND PROBLEMS

Section 12.2

19. Suppose a future scientist explores a distant planet and discovers a novel form of double-stranded nucleic acid. When this nucleic acid is exposed to DNA polymerases from *E. coli*, replication takes place continuously on both strands. What conclusion can you draw about the structure of this novel nucleic acid?

20. Phosphorus is required to synthesize the deoxyribonucleoside triphosphates used in DNA replication. A geneticist grows some *E. coli* in a medium containing nonradioactive phosphorus for many generations. A sample of the bacteria is then transferred to a medium that contains a radioactive isotope of phosphorus (^{32}P). Samples of the bacteria are removed immediately after the transfer and after one and two rounds of replication. Assume that newly synthesized DNA contains ^{32}P and the original DNA contains nonradioactive phosphorus. What will be the distribution of radioactivity in the DNA of the bacteria in each sample? Will radioactivity be detected in neither, one, or both strands of the DNA?

21.

*A line of mouse cells is grown for many generations in a medium with ^{15}N. Cells in G_1 are then switched to a new medium that contains ^{14}N. Draw a pair of homologous chromosomes from these cells at the following stages, showing the two strands of DNA molecules found in the chromosomes (see Chapter 2 for a review of chromosomes in different stages of the cell cycle). Use different colors to represent strands with ^{14}N and ^{15}N.

    a. Cells in G_1, before switching to medium with ^{14}N
    b. Cells in G_2, after switching to medium with ^{14}N
    c. Cells in anaphase of mitosis, after switching to medium with ^{14}N
    d. Cells in metaphase I of meiosis, after switching to medium with ^{14}N

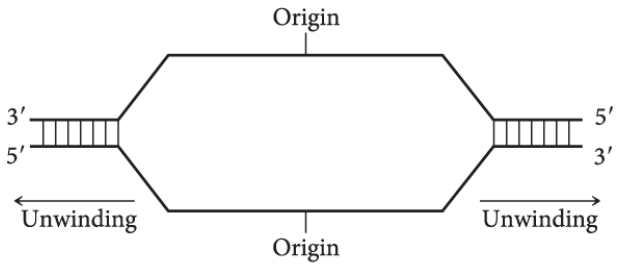
e. Cells in anaphase II of meiosis, after switching to medium with ^{14}N

22. *A circular molecule of DNA contains 1 million base pairs. If the rate of DNA synthesis at a replication fork is 100,000 nucleotides per minute, how much time will theta replication require to completely replicate the molecule, assuming that theta replication is bidirectional? How long will replication of this circular chromosome by rolling-circle replication take? Ignore replication of the displaced strand in rolling-circle replication.

23. A bacterium synthesizes DNA at each replication fork at a rate of 1000 nucleotides per second. If this bacterium completely replicates its circular chromosome by theta replication in 30 minutes, how many base pairs of DNA does its chromosome contain?

Section 12.3

24. *The following diagram represents a DNA molecule that is undergoing replication. Draw in the strands of newly synthesized DNA and identify (a) the polarity of the newly synthesized strands, (b) leading and lagging strands, (c) Okazaki fragments, and (d) RNA primers.



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Description
The top strand has 3 prime end on the left side and the bottom strand has 3 prime end on the right side. In the middle a replication bubble is formed with origin labeled in the center. The left end shows an arrow pointing towards left and is labeled unwinding. The right end shows an arrow pointing towards right and is labeled unwinding.

25. In **Figure 12.8**, which is the leading strand and which is the lagging strand?

26. *What would be the effect on DNA replication of mutations that destroyed each of the following activities of DNA polymerase I?
    a. 3′→5′ exonuclease activity
    b. 5′→3′ exonuclease activity
    c. 5′→3′ polymerase activity

27. Which of the DNA polymerases shown in **Table 12.3** have the ability to proofread?

28. How would DNA replication be affected in a bacterial cell that is lacking DNA gyrase?

29. *If the gene for primase were mutated so that no functional primase was produced, what would be the effect on theta

replication? On rolling-circle replication?

Section 12.4

30. Eukaryotic licensing factors prevent DNA replication from being initiated at origins more than once in the cell cycle. After replication has begun at an origin, a protein called Geminin inhibits licensing factors that are required for MCM2-7 to bind to an origin and initiate replication. Thus, when Geminin is present, MCM2-7 will not bind to an origin. At the end of mitosis, Geminin is degraded, allowing MCM2-7 to bind once again to DNA and relicense the origin. Marina Melixetian and her colleagues suppressed the expression of Geminin protein in human cells by treating the cells with small interfering RNAs (siRNAs) complementary to Geminin messenger RNA (M. Melixetian et al. 2004. *Journal of Cell Biology* 165:473–482). (Small interfering RNAs form a complex with proteins and pair with complementary sequences on mRNAs; the complex then cleaves the mRNA, so there is no translation of the mRNA; see Chapter 14.) Forty-eight hours after treatment with siRNA, the Geminin-depleted cells were enlarged and contained a single giant nucleus. Analysis of DNA content showed that many of these Geminin-depleted cells were 4n or greater. Explain these results.

31. *What results would be expected in the experiment outlined in **Figure 12.16** if, during replication, all the original histone proteins remained on one strand of the DNA and new histones attached to the other strand?

32. A number of scientists who study cancer treatment have become interested in telomerase. Why? How might anticancer therapies that target telomerase work? (See Chapter 23 on cancer genetics.)

33. *The enzyme telomerase is part protein and part RNA. What would be the most likely effect of a large deletion in the gene that encodes the RNA component of telomerase? How would the function of telomerase be affected?

34. Dyskeratosis congenita (DKC) is a rare genetic disorder characterized by abnormal fingernails and skin pigmentation, the formation of white patches on the tongue and cheek, and progressive failure of the bone marrow. An autosomal dominant form of DKC results from mutations in the gene that encodes the RNA component of telomerase. Tom Vulliamy and his colleagues examined a series of families with autosomal dominant DKC (T. Vulliamy et al. 2004. *Nature Genetics* 36:447–449). They observed that the median age of onset of DKC in parents was 37 years, whereas the median age of onset in the children of affected parents was 14.5 years. Thus, DKC in these families arose at progressively younger ages in successive generations, a phenomenon known as anticipation (see Chapter 5). The researchers measured the telomere lengths of members of these families; the measurements are given in the accompanying table. Telomeres normally shorten with age,

so telomere length was adjusted for age; the values given in the table are the differences between the actual length and the expected length based on age. Note that the values of all members of these families are negative, indicating that their telomeres are shorter than normal for their age; the more negative the number, the shorter the telomere.

Parent telomere length	Child telomere length
−4.7	−6.1
	−6.6
	−6.0
−3.9	−0.6
−1.4	−2.2
−5.2	−5.4
−2.2	−3.6
−4.4	−2.0
−4.3	−6.8
−5.0	−3.8
−5.3	−6.4

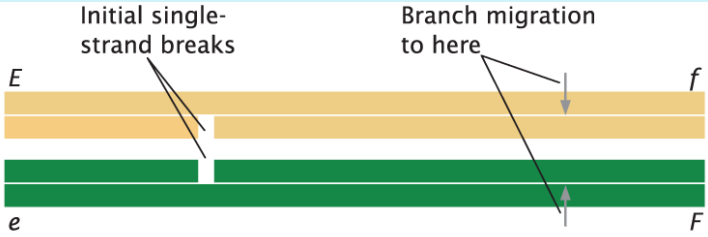
−0.6	−2.5
−1.3	−5.1
	−3.9
−4.2	−5.9

Note: The telomere lengths given in the table are the differences between the actual length and the expected length based on age. Negative values indicate shorter than expected telomeres.

a. How does the telomere length of the parents compare with the telomere length of the children? (Hint: Calculate the average telomere length of all parents and the average telomere length of all children.)
b. Explain why the telomeres of people with DKC are shorter than normal.
c. Explain why DKC arises at an earlier age in subsequent generations.

35. CONNECT An individual is heterozygous at two loci (*Ee Ff*), and the two genes are in repulsion (see pp. 187–188 in Chapter 7). Assume that single-strand breaks and branch migration occur at the positions shown at right. Using different colors to represent the two homologous chromosomes, draw the noncrossover recombinant and crossover recombinant DNA

molecules that will result from homologous recombination. (Hint: See Figure 12.19.)



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Description

The top chromosome is yellow and has big E small F genes. The bottom chromosome is green and has small E big F genes. For the yellow D N A molecule, the big E locus is on the far left, one-third to the right is the single-stranded breaking point, one-third more to the right is an arrow pointing the branch migration point, and at the very end is the small F locus. For the green D N A molecule, small E focus is on far left, one-third to the right on the bottom D N A strand is the single-stranded breaking point, one-third more to the right is an arrow pointing the branch migration point, and at the very end is the big F locus.

CHALLENGE QUESTIONS

Section 12.3

36. A conditional mutation expresses its mutant phenotype only under certain conditions (the restrictive conditions) and expresses the normal phenotype under other conditions (the

permissive conditions). One type of conditional mutation is a temperature-sensitive mutation, which expresses the mutant phenotype only at certain temperatures.

Strains of *E. coli* have been isolated that contain temperature-sensitive mutations in genes encoding different components of the replication machinery. In each of these strains, the protein produced by the mutated gene is nonfunctional under the restrictive conditions. You grow these strains under the permissive conditions and then abruptly switch them to the restrictive conditions. After one round of replication under the restrictive conditions, you isolate DNA from each strain and analyze it. What characteristics would you expect to see in the DNA isolated from a strain with a temperature-sensitive mutation in the gene that encodes each of the following proteins?

a. DNA ligase
b. DNA polymerase I
c. DNA polymerase III
d. Primase
e. Initiator protein

Section 12.4

37.

DNA topoisomerases play important roles in DNA replication and in supercoiling (see Chapter 11). These enzymes are also the targets for certain anticancer drugs. Eric Nelson and his colleagues studied m-AMSA, one of the anticancer

compounds that acts on topoisomerase (E. M. Nelson, K. M. Tewey, and L. F. Liu. 1984. *Proceedings of the National Academy of Sciences of the United States of America* 81:1361–1365). They found that m-AMSA stabilizes an intermediate produced in the course of topoisomerase action. The intermediate consists of topoisomerase bound to the broken ends of the DNA. Breaks in DNA that are produced by anticancer compounds such as m-AMSA inhibit the replication of the cellular DNA and thus stop cancer cells from proliferating. Explain how m-AMSA and other anticancer agents that target topoisomerase enzymes taking part in replication might lead to DNA breaks and chromosome rearrangements.

38. * CONNECT The regulation of replication is essential to genomic stability. Normally, the DNA is replicated just once in every eukaryotic cell cycle (in the S phase). Normal cells produce protein A, which increases in concentration in the S phase. In cells that have a mutated copy of the gene encoding protein A, the protein is not functional, and replication takes place continuously throughout the cell cycle, with the result that cells may have 50 times the normal amount of DNA. Protein B is normally present in G_1 but disappears from the cell nucleus during the S phase. In cells with a mutated copy of the gene encoding protein A, the levels of protein B fail to drop in the S phase and, instead, remain high throughout the cell cycle. When the gene for protein B is mutated, no replication takes place. Propose a mechanism for how protein A and protein B might

normally regulate replication so that each cell gets the proper amount of DNA. Explain how mutation of these genes produces the effects just described.

🗣️ ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 12.1

1. In the 1996 movie *Multiplicity*, Doug (played by Michael Keaton) is a construction worker who wants to spend more time with his family. He meets a friendly scientist who has developed a method for cloning humans. Doug decides to make a clone of himself who can take over his work while he spends quality time with his family. The clone, named Two, seems great at first, but problems surface in his functioning. Two decides to make a clone of himself, so he won't have to work, and creates Three. A fourth clone is eventually made. Each successive clone seems to have more problems. Ignore, for the present, the technical difficulties and ethical problems with making an instantaneous adult clone of a human. Is there any genetic validity to the premise that making a clone of a clone might create problems? If so, what might those problems be?

Section 12.2

2. In their experiment, could Meselson and Stahl have used two different isotopes of carbon instead of ^{14}N and ^{15}N? Why or why not? What about two different isotopes of sulfur?

Section 12.3

3. DNA polymerases cannot act as primers for replication, yet primase and other RNA polymerases can. Some geneticists have speculated that the inability of DNA polymerase to prime replication is a result of its proofreading function. This hypothesis argues that proofreading is essential for the faithful transmission of genetic information and that because DNA polymerases have evolved the ability to proofread, they cannot prime DNA synthesis. Explain why proofreading and priming functions in the same enzyme might be incompatible.

Section 12.4

4. Many anticancer drugs work by interfering with different components of the replication machinery. For example, camptothecin inhibits the action of topoisomerase during replication. These compounds often have unwanted side effects. What are some common negative side effects of chemotherapy, and why do they arise?

5. Okazaki fragments in bacterial cells are 1000 to 2000 nucleotides in length. Those in eukaryotic cells are much shorter, usually only 100 to 200 nucleotides in length. Propose some possible explanations for why Okazaki fragments in bacteria are longer

than those in eukaryotes. What difference between the two cell types could lead to the difference in Okazaki fragment length?

6. HeLa cells are a line of cells grown in laboratory culture that has been used extensively in research. This cell line was originally derived from malignant cervical cancer cells that were removed from a woman named Henrietta Lacks in 1951. They were subsequently grown in culture and shipped to research laboratories around the world, where they have been used in many important experiments. Like HeLa cells, many other cell lines were originally taken from cancerous tissue. Why are cancer cells often used for developing cell lines?

7. For centuries, people have searched for the fabled Fountain of Youth, said to confer the ability to forestall old age and remain young forever. To gain insight into how tackling aging by targeting telomerase might work, researchers looked at mice that lack a telomerase gene. These mice have shorter telomeres than normal mice and age prematurely. But when these mice are engineered to express telomerase in their somatic cells, their telomeres lengthen, and the effects of aging are reversed. This observation suggests that a drug that stimulates the expression of telomerase in somatic cells could prevent telomere shortening and stop the aging process. Do you think such a drug would work well in humans? What might be some potential side effects of such a drug?

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Create a concept map that outlines the connection between origins of replication and Meier-Gorlin syndrome using the following main concepts: eukaryotic cells, large genome, numerous origins of replication, replication once per cell cycle, licensed replication, Meier-Gorlin syndrome, small stature, small ears, no kneecaps, origin-replication complex.

Sections 12.2 and 12.3

2. Outline the process of DNA replication in bacteria by drawing a concept map that includes the following terms: replication, origin of replication, initiator protein, replication bubble, continuous replication, discontinuous replication, leading strand, lagging strand, Okazaki fragments, DNA ligase, helicase, single-strand DNA, replication fork, single-strand binding proteins, DNA gyrase, primase, RNA primer, DNA polymerase I, DNA polymerase III.

Section 12.4

3. Draw a concept map that connects important concepts across Chapters 2 and 12: DNA replication, G_1/S checkpoint, interphase, S phase, sister chromatids, anaphase of mitosis,

semiconservative replication, $5' \rightarrow 3'$, continuous replication, discontinuous replication, lagging strand, leading strand.

4. Create a concept map that summarizes the differences in bacterial and eukaryotic replication.
5. Show the relationships among the following concepts by drawing a concept map: end of replication problem, telomeres, telomerase, G-rich strand, RNA primer, $3'$-OH, somatic cells, germ cells, end of chromosome, telomeric repeats, aging, cancer cells, limited cell divisions, telomere shortening.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com

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CHAPTER 13

Transcription

Frank Krahmer/Getty Images.

DNA is a single-lane highway on which DNA and RNA polymerases race at high speeds, carrying out replication and transcription. Cells have evolved mechanisms to prevent and fix collisions that inevitably occur between these molecules.

Wrecks on the DNA Highway

DNA is a heavily used single-lane highway, in which DNA and RNA polymerases race at breakneck speeds, carrying out the vital processes of replication and transcription. And like cars traveling a

single-lane road, conflicts and collisions are inevitable. How do cells avoid these conflicts and deal with collisions?

As we discussed in Chapter 12, replication is the mechanism by which new DNA molecules are synthesized using single-stranded DNA templates through semiconservative replication. DNA synthesis is integral to cell division: before a cell can divide, it must copy its entire genome so that each cell resulting from division receives a complete set of genetic instructions. Transcription, discussed in this chapter, is equally important. It is the first step that uses the coding instructions in the DNA to create the traits of an organism, through the copying of DNA sequences into an RNA molecule.

Both replication and transcription require reading DNA polynucleotide strands. Although they begin at different places (replication begins at origins and transcription starts at promoters), both processes move in the same $5' \rightarrow 3'$ direction and can take place on either DNA strand, so encounters between DNA and RNA polymerases are sure to occur. These enzymes travel at remarkable speeds: replication in bacteria takes place at 1000 nucleotides per second; transcription is slower, but still incredibly fast, occurring at about 40 nucleotides per second. If these two processes meet head on, catastrophic collisions occur. Even when traveling in the same direction, differences in the speed of replication and transcription lead to rear-end crashes, like collisions between cars traveling at 10

mph and others traveling at 250 mph, all on the same single-lane road.

Head-on collisions between DNA and RNA polymerases usually bring a halt to replication and cause the premature termination of transcription. Replication must then be restarted for the entire genome to be copied. Similarly, transcription of individual genes must be reinitiated. Collisions also lead to DNA breaks and mutations, which cause genetic diseases, cancer, and other problems. Because of these negative consequences, cells have evolved a number of mechanisms to avoid, tolerate, and fix collisions between replication and transcription.

In bacteria, replication usually begins at a single origin, occurs continuously on both strands, and is not segregated in time from transcription, so conflicts between the two processes are common. Many bacteria have evolved in a simple way to avoid head-on collisions: highly transcribed and essential genes are transcribed in the same direction that replication occurs, so head-on clashes are avoided. For example, in the bacterium *Bacillus subtilis*, 94% of essential genes are transcribed in the same direction that replication occurs. Although codirectional replication and transcription avoid head-on collisions, this does not prevent rear-end wrecks, as the faster replication complexes overtake and ram into the slower-moving transcription enzymes. To deal with crashes, bacteria have evolved mechanisms to restart replication

forks stalled by collisions, remove transcription complexes blocked by collisions, and repair DNA breaks and mutations resulting from collisions.

For many years, researchers assumed that collisions would be less common in eukaryotic cells. Replication in eukaryotes is restricted to S phase (see Chapter 2), allowing transcription to occur without risk of colliding with replication complexes during other phases of the cell cycle. Also, the speeds of replication and transcription complexes in eukaryotes are more similar, reducing the risk of rear-end collisions. Nevertheless, research now shows that collisions between replication and transcription do occur in eukaryotes, and eukaryotes, like bacteria, have evolved mechanisms to avoid and correct damage caused by collisions. For example, gene expression that must take place in S phase is controlled so that transcription occurs in early S phase for genes that are replicated in late S phase, and vice versa. Key to the controlling conflicts between replication and transcription is the efficient and highly organized manner in which transcription occurs (discussed in this chapter) and the careful control of gene expression (discussed in Chapters 16 and 17).

THINK-PAIR-SHARE

- In bacteria, RNA polymerases carry out transcription more slowly than DNA polymerases carry out replication. Why is speed more important in replication than in transcription?

Collisions between replication and transcription complexes are just one of the many challenges created by the need to faithfully transcribe a DNA template into an RNA molecule. This chapter is about the process of transcription, the first step in the pathway of information transfer from DNA (genotype) to protein (phenotype). Transcription is a complex process that requires a DNA template, precursors to RNA nucleotides, and a number of protein components. As we examine the stages of transcription, try to keep all the details in perspective and focus on understanding how they relate to the overall purpose of transcription: the selective synthesis of an RNA molecule.

This chapter begins with a brief review of RNA structure and a discussion of the different classes of RNA. We then consider the major components required for transcription. Finally, we explore the process of transcription. At several points in the text, we pause to consider some general principles that emerge.

13.1 RNA, Consisting of a Single Strand of Ribonucleotides, Participates in a Variety of Cellular Functions

Before we begin our study of transcription, let's consider the past and present importance of RNA, review the structure of RNA, and examine some of the different types of RNA molecules.

An Early RNA World

Life requires two basic functions. First, living organisms must be able to store and faithfully transmit genetic information during reproduction. Second, they must have the ability to catalyze the chemical transformations that drive life processes. A long-held belief was that the functions of information storage and chemical transformation are handled by two entirely different types of molecules: genetic information is stored in nucleic acids, whereas chemical transformations are catalyzed by protein enzymes. This biochemical dichotomy created a dilemma: Which came first, proteins or nucleic acids? If nucleic acids carry the coding instructions for proteins, how could proteins be generated without them? Nucleic acids are unable to copy themselves, so how could they be generated without proteins? If DNA and proteins each require the other, how could life begin?

This apparent paradox was answered in 1981 when Thomas Cech and his colleagues discovered that RNA can serve as a biological catalyst. They found that some RNA molecules from the protozoan *Tetrahymena thermophila* can excise 400 nucleotides from its RNA in the absence of any protein. Other examples of catalytic RNAs have now been discovered in different types of cells. Called **ribozymes**, these catalytic RNA molecules can cut out parts of their own sequences, connect some RNA molecules together, replicate others, and catalyze the formation of peptide bonds between amino acids. The discovery of ribozymes complements other evidence suggesting that the original genetic material was RNA.

Self-replicating ribozymes probably first arose between 3.5 billion and 4 billion years ago and may have begun the evolution of life on Earth. Early life was probably an RNA world, where RNA molecules served both as carriers of genetic information and as catalysts that drove the chemical reactions needed to sustain and perpetuate life. These catalytic RNAs may have acquired the ability to synthesize protein-based enzymes, which are more efficient catalysts. With enzymes taking over more and more of the catalytic functions, RNA probably became relegated to the role of information storage and transfer. DNA, with its chemical stability and faithful replication, eventually replaced RNA as the primary carrier of genetic information. Nevertheless, RNA is either produced by or plays a vital role in many biological processes, including transcription, replication, RNA processing, and translation. Research in the past 20 years has also determined that newly discovered small RNA molecules play a fundamental role in many basic biological processes, demonstrating that life today is still very much an

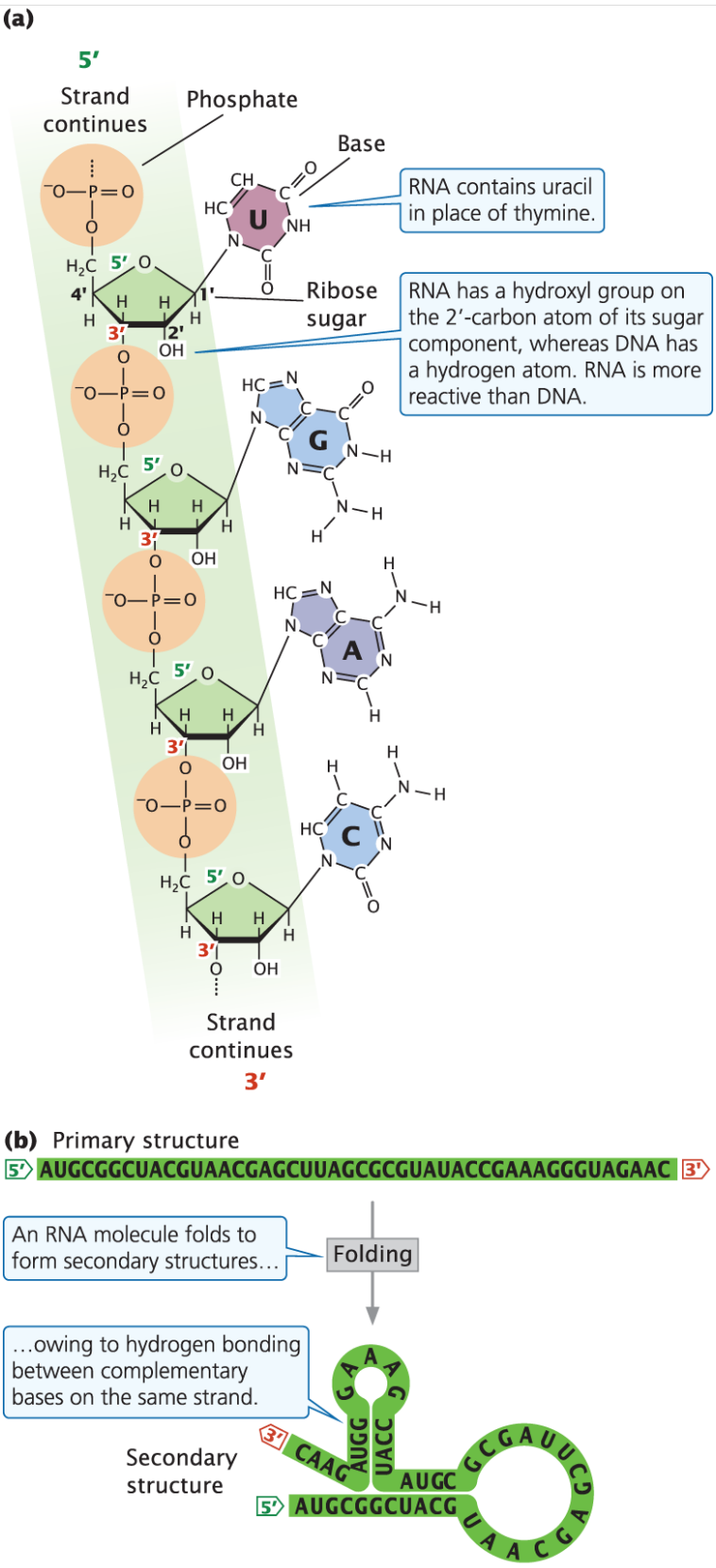
RNA world. These small RNA molecules will be discussed in more detail in Chapter 14.

CONCEPTS

Early in the history of life, RNA probably served both as the original genetic material and as biological catalysts.

The Structure of RNA

RNA, like DNA, is a polymer of nucleotides, each consisting of a sugar, a phosphate group, and a nitrogenous base, joined together by phosphodiester bonds (see Chapter 10). However, there are several important differences in the structures of DNA and RNA. Whereas DNA nucleotides contain deoxyribose sugars, RNA nucleotides have ribose sugars (**Figure 13.1a**). With a free hydroxyl group on the 2′-carbon atom of the ribose sugar, RNA is degraded rapidly under alkaline conditions. The deoxyribose sugar of DNA lacks this free hydroxyl group, so DNA is a more stable molecule. Another important difference is that the pyrimidine uracil is present in RNA instead of thymine, one of the two pyrimidines found in DNA.



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13.1 RNA has a primary and a secondary structure.

Description
Diagram a, at the top, shows an R N A strand from 5 prime to 3 prime. A phosphate group is bonded to C 5 prime of a sugar molecule while a uracil base is bonded to its C 1 prime. A phosphate group attached at C 3 prime bonds to the C 5 prime of next sugar molecule. A caption reads, R N A contains uracil in place of thymine. R N A has a hydroxyl group on the 2 prime carbon atom of its sugar component, whereas D N A has a hydrogen atom. R N A is more reactive than D N A. Diagram b, below labeled primary structure, shows a strand from 5 prime to 3 prime. The nucleotides are as follows: A, U, G, C, G, G, C, U, A, C, G, U, A, A, C, G, A, G, C, U, U, A, G, C, G, C, G, U, A, U, A, C, C, G, A, A, A, G, G, G, U, A, G, A, A, C. Folding occurs below. A caption reads, an R N A molecule folds to form secondary structures owing to hydrogen bonding between complementary bases on the same strand. The structure formed after folding has double-stranded stems and single-stranded loops. The first loop has the following sequence: G, A, A, A, G and the second loop has the following sequence: G, C, G, A, U, U, C, G, A, G, C, A, A, U.

A final difference in the structures of DNA and RNA is that RNA usually consists of a single polynucleotide strand, whereas DNA normally consists of two polynucleotide strands joined by hydrogen bonding between complementary bases (although some viruses contain double-stranded RNA genomes, as discussed in Chapter 9). Although RNA is usually single stranded, short complementary regions within a nucleotide strand can pair and form secondary structures (**Figure 13.1b**). These RNA secondary structures are often called hairpins (or hairpin-loop or stem-loop structures). When two regions within a single RNA molecule pair up, the strands in those regions must be antiparallel and with pairing between cytosine and guanine and between adenine and uracil (although occasionally guanine pairs with uracil).

The formation of secondary structures plays an important role in RNA function. Secondary structure is determined by the base sequence of the nucleotide strand, so different RNA molecules can assume different structures. Because their structure determines their function, RNA molecules have the potential for tremendous variation in function. With its two complementary strands forming a helix, DNA is much more restricted in the range of secondary structures that it can assume and so has fewer functional roles in the cell. Similarities and differences in DNA and RNA structures are summarized in Table 13.1.

› TRY PROBLEM 15

TABLE 13.1 Comparison of DNA and RNA structures

Characteristic	DNA	RNA
Composed of nucleotides	Yes	Yes
Type of sugar	Deoxyribose	Ribose
Presence of 2′-OH group	No	Yes
Bases	A, G, C, T	A, G, C, U
Nucleotides joined by phosphodiester bonds	Yes	Yes
Double or single stranded	Usually double	Usually single
Secondary structure	Double	Many

	helix	types
Stability	Stable	Easily degraded

Classes of RNA

RNA molecules perform a variety of functions in the cell. **Ribosomal RNA (rRNA)** and ribosomal protein subunits make up the ribosome, the site of protein assembly. We'll take a more detailed look at the ribosome in Chapter 14. **Messenger RNA (mRNA)** carries the coding instructions for a polypeptide chain from DNA to a ribosome. After attaching to the ribosome, an mRNA molecule specifies the sequence of the amino acids in a polypeptide chain and provides a template for the joining of those amino acids. Large precursor molecules, which are termed **pre-messenger RNAs (pre-mRNAs)**, are the immediate products of transcription in eukaryotic cells. Pre-mRNAs are modified extensively before becoming mRNA and exiting the nucleus for translation into protein. Bacterial cells do not possess pre-mRNA; in these cells, transcription takes place concurrently with translation.

Transfer RNA (tRNA) serves as the link between the coding sequence of nucleotides in an mRNA molecule and the amino acid sequence of a polypeptide chain. Each tRNA attaches to one particular type of amino acid and helps incorporate that amino acid into a polypeptide chain (as described in Chapter 15).

Additional classes of RNA molecules are found in the nuclei of eukaryotic cells. **Small nuclear RNAs (snRNAs)** combine with small protein subunits to form **small nuclear ribonucleoproteins** (snRNPs, affectionately known as "snurps"). Some snRNAs participate in the processing of RNA, converting pre-mRNA into mRNA. **Small nucleolar RNAs (snoRNAs)** take part in the processing of rRNA.

Two types of very small and abundant RNA molecules found in the cytoplasm of eukaryotic cells, termed **microRNAs (miRNAs)** and **small interfering RNAs (siRNAs)**, carry out RNA interference (RNAi), a process in which these small RNA molecules help trigger the degradation of mRNA or inhibit its translation into protein. More will be said about RNA interference in Chapter 14. Another class of small RNA molecules are **Piwi-interacting RNAs** (**piRNAs**; named after Piwi proteins, with which they interact). Found in mammalian testes, these RNA molecules are similar to miRNAs and siRNAs; they have a role in suppressing the expression of transposable elements (see Chapter 18) in reproductive cells. **Long noncoding RNAs (lncRNAs)** are relatively long RNA molecules found in eukaryotes that do not code for proteins. They provide a variety of functions, including regulation of gene expression. In prokaryotes, an RNA interference-like system has been discovered, in which small **CRISPR RNAs (crRNAs)** assist in the destruction of foreign DNA molecules. Some of the different classes of RNA molecules are summarized in **Table 13.2**.

TABLE 13.2 Locations and functions of different classes of RNA molecules

Class of RNA	Cell Type	Location of Function in Eukaryotic Cells*	Function
Ribosomal RNA (rRNA)	Prokaryotic and eukaryotic	Cytoplasm	Structural and functional components of the ribosome
Messenger RNA (mRNA)	Prokaryotic and eukaryotic	Nucleus and cytoplasm	Carries genetic code for proteins
Transfer RNA (tRNA)	Prokaryotic and eukaryotic	Cytoplasm	Helps incorporate amino acids into polypeptide chain
Pre-messenger RNA (pre-mRNA)	Eukaryotic	Nucleus	Precursor to mRNA

Small nuclear RNA (snRNA)	Eukaryotic	Nucleus	Processing of pre-mRNA
Small nucleolar RNA (snoRNA)	Eukaryotic	Nucleus	Processing and assembly of rRNA
MicroRNA (miRNA)	Eukaryotic	Nucleus and cytoplasm	Inhibits translation of mRNA
Small interfering RNA (siRNA)	Eukaryotic	Nucleus and cytoplasm	Triggers degradation of other RNA molecules
Piwi-interacting RNA (piRNA)	Eukaryotic	Nucleus and cytoplasm	Suppresses the transcription of transposable elements in reproductive cells
Long noncoding	Eukaryotic	Nucleus and	Variety of functions

RNA (lncRNA)		cytoplasm	
CRISPR RNA (crRNA)	Prokaryotic	—	Assists destruction of foreign DNA

*All eukaryotic RNAs are synthesized in the nucleus.

CONCEPTS

RNA differs from DNA in that RNA possesses a hydroxyl group on the 2′-carbon atom of its sugar, contains uracil instead of thymine, and is usually single stranded. Several classes of RNA exist within bacterial and eukaryotic cells.

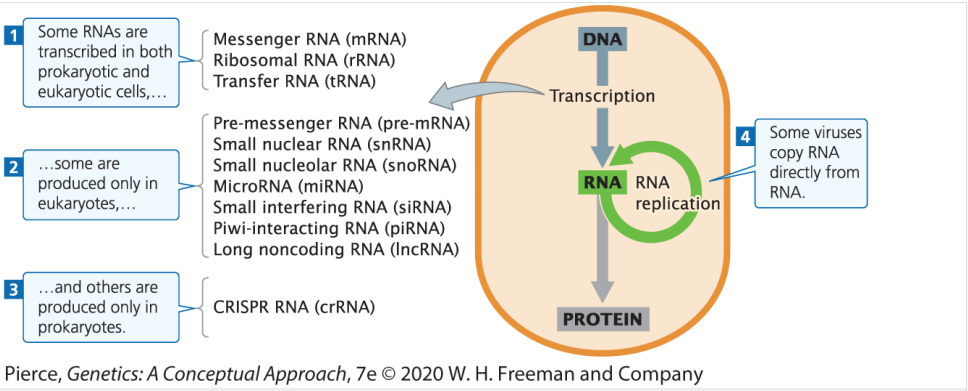
✔ CONCEPT CHECK 1

Which class of RNA is correctly paired with its function?

a. Small nuclear RNA (snRNA): processes rRNA
b. Transfer RNA (tRNA): attaches to an amino acid
c. MicroRNA (miRNA): carries information for the amino acid sequence of a protein
d. Ribosomal RNA (rRNA): carries out RNA interference

13.2 Transcription Is the Synthesis of an RNA Molecule from a DNA Template

All cellular RNAs are synthesized from DNA templates through the process of transcription (Figure 13.2). Transcription is in many ways similar to the process of replication, but a fundamental difference relates to the length of the template used. In replication, all the nucleotides in the DNA molecule are copied, but in transcription, only parts of the DNA molecule are transcribed into RNA. Because not all gene products are needed at the same time or in the same cell, the constant transcription of all of a cell's genes would be highly inefficient. Furthermore, much of the DNA does not encode any functional product, and transcription of such sequences would be pointless. Transcription is, in fact, a highly selective process: individual genes are transcribed only as their products are needed. However, this selectivity imposes a fundamental problem on the cell: how to recognize individual genes and transcribe them at the proper time and place.



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13.2 All cellular types of RNA are transcribed from DNA.

Description

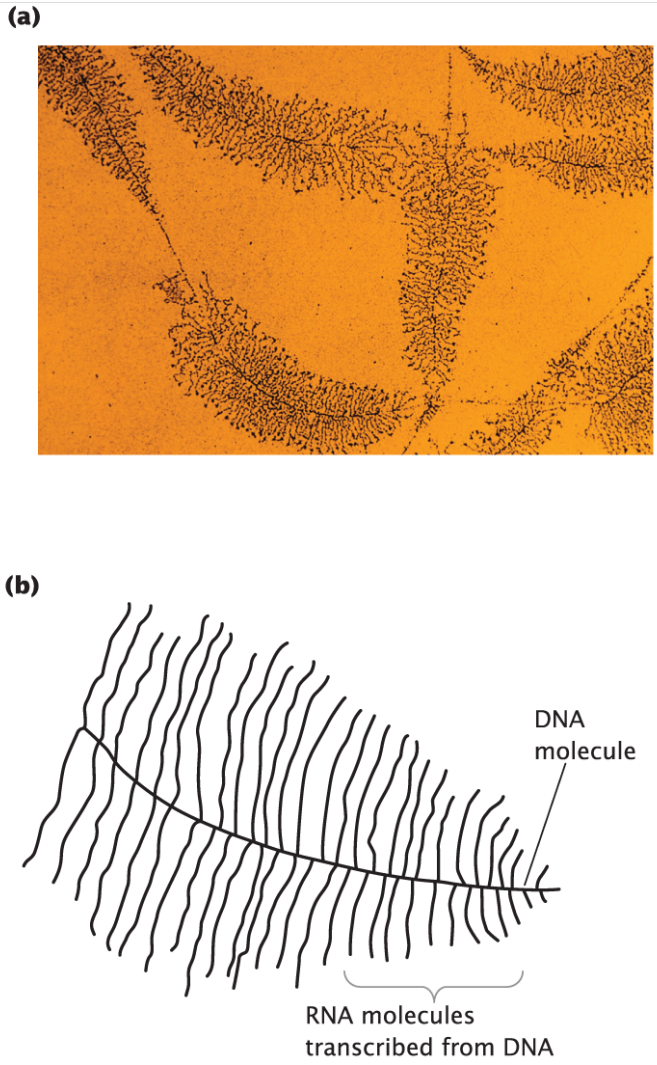
The illustration shows a cell where D N A undergoes transcription. A caption reads, some R N As are transcribed in both prokaryotic and eukaryotic cells. These R N As include messenger R N A (m R N A), ribosomal R N A (r R N A), and transfer R N A (t R N A). Another caption reads, Some are produced only in eukaryotes. These include pre-messenger R N A (pre-m R N A), small nuclear R N A (s n R N A), small nucleolar R N A (s n o R N A), micro R N A (m i R N A), small interfering R N A (s i R N A), piwi-interacting R N A (p i R N A), and long noncoding R N A (l n c R N A). A caption reads, and others are produced only in prokaryotes. These include C R I S P R R N A (c r R N A). After transcription, R N A is produced. R N A undergoes replication to produce R N A again. A caption reads, some viruses copy R N A directly from R N A. R N A eventually leads to protein formation.

Like replication, transcription requires three major components:

1. A DNA template
2. The raw materials (ribonucleotide triphosphates) needed to build a new RNA molecule
3. The transcription apparatus, consisting of the proteins necessary for catalyzing the synthesis of RNA

The Template

In 1970, Oscar Miller, Jr.; Barbara Hamkalo; and Charles Thomas used electron microscopy to demonstrate that RNA is transcribed from a DNA template. They broke open salamander oocytes, extracted chromatin, and spread the chromatin onto a fine-mesh grid. Under the electron microscope, they observed Christmas-tree-like structures, each consisting of a thin central fiber (the trunk of the tree) to which were attached strings (the branches) bearing granules (**Figure 13.3a**). The addition of deoxyribonuclease (an enzyme that degrades DNA) caused the central fibers to disappear, indicating that the "tree trunks" were DNA molecules. Ribonuclease (an enzyme that degrades RNA) removed the granular strings, indicating that the branches were RNA. Their conclusion was that each "Christmas tree" represents a gene undergoing transcription (**Figure 13.3b**). The transcription of each gene begins at the top of the tree; there, little of the DNA has been transcribed, and the RNA branches are short. As the transcription apparatus moves down the tree, transcribing more of the template, the RNA molecules lengthen, producing the long branches at the bottom.



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Part a: Medical Images RM/Phototake, Inc.

13.3 Under the electron microscope, DNA molecules undergoing transcription exhibit Christmas-tree-like structures. (a) Electron micrograph of transcription units. (b) The trunk of each "Christmas tree" (a transcription unit) represents a portion of a DNA molecule; the tree branches are RNA molecules that have been transcribed from the DNA. As the transcription apparatus moves down the DNA, transcribing more of the template, the RNA molecules become longer and longer.

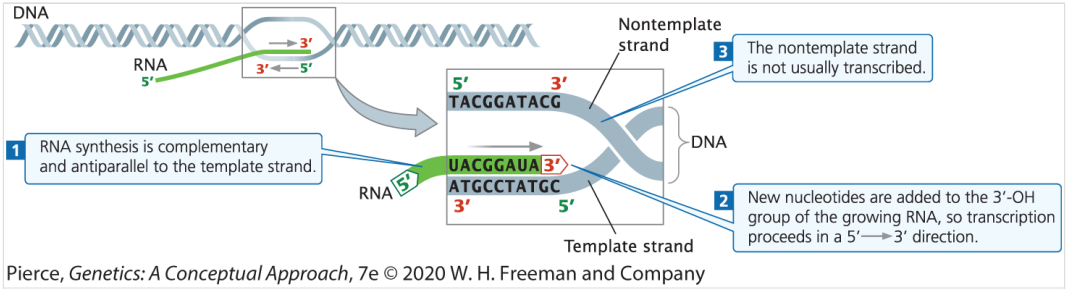
Description

The electron micrograph a shows various randomly arranged, black colored, transcription units that appear like miniature Christmas trees over an orange background. The drawing

b below shows a Christmas tree where the trunk represents a D N A molecule and the branches represent R N A molecules.

THE TRANSCRIBED STRAND

The template for RNA synthesis, as for DNA synthesis, is a single strand of the DNA double helix. Unlike replication, however, the transcription of a gene takes place on only one of the two nucleotide strands of DNA (Figure 13.4). The nucleotide strand used for transcription is termed the **template strand**. The other strand, called the **nontemplate strand**, is not ordinarily transcribed. Thus, within a gene, only one of the nucleotide strands is normally transcribed into RNA (although there are some exceptions to this rule).



13.4 Transcription produces RNA molecules that are complementary and antiparallel to one of the two nucleotide strands of DNA: the template strand.

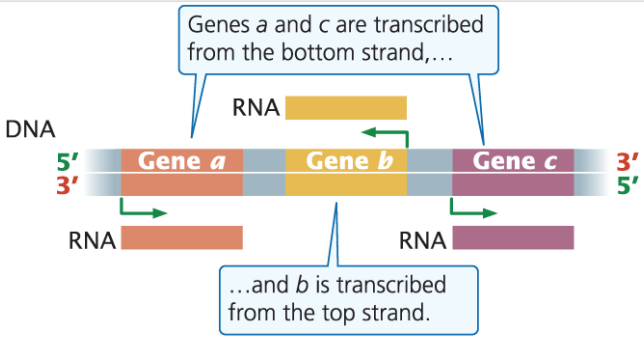
Description

The illustration shows a double helical D N A strand, at the top, with a replication bubble. A newly synthesized R N A is produced from 5 prime to 3 prime while the template strand is from 3 prime to 5 prime. The nucleotides of the newly synthesized R N A strand are: U, A, C, G, G, A, U, A which is complementary to the nucleotides on the template strand (A, T, G, C, C, T, A, T). A magnified view, on the right, shows the newly synthesized strand formed above the template D N A strand. A caption reads, R N A synthesis is complementary and

antiparallel to the template strand. New nucleotides are added to the 3 prime O H group of the growing R N A; so transcription proceeds in a 5 prime to 3 prime direction. A caption reads, The nontemplate strand is not usually transcribed.

During transcription, an RNA molecule that is complementary and antiparallel to the DNA template strand is synthesized (see Figure 13.4). The RNA transcript has the same polarity and base sequence as the nontemplate strand except that it contains U rather than T. In most organisms, each gene is transcribed from a single DNA strand, but different genes may be transcribed from different strands, as shown in **Figure 13.5**. ❯ **TRY PROBLEM 16**

THINK-PAIR-SHARE Question 1



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13.5 RNA is transcribed from one DNA strand. In most organisms, each gene is transcribed from a single DNA strand, but different genes may be transcribed from either DNA strand.

Description
The illustration shows a double stranded D N A molecule with genes small A, small B, and small C. Genes small A and small C are transcribed from the bottom strand that runs from

3 prime to 5 prime. These genes are transcribed from left to right. Gene small B is transcribed from top strand that runs from 5 prime to 3 prime. This gene is transcribed from right to left. A caption reads, Genes small A and small C are transcribed from the bottom strand and small B is transcribed from the top strand.

CONCEPTS

Within a single gene, only one of the two DNA strands, the template strand, is usually transcribed into RNA.

✔ CONCEPT CHECK 2

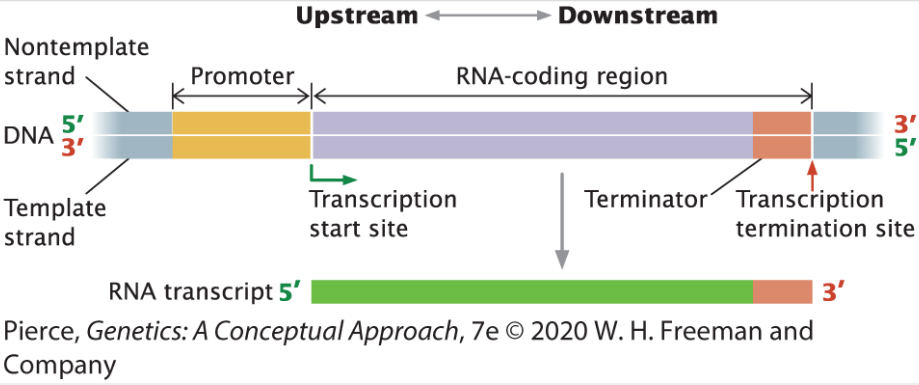
What is the difference between the template strand and the nontemplate strand?

THE TRANSCRIPTION UNIT

A **transcription unit** is a stretch of DNA that encodes an RNA molecule and the sequences necessary for its transcription. How does the complex of enzymes and proteins that performs transcription—the transcription apparatus—recognize a transcription unit? How does it know which DNA strand to read and where to start and stop? This information is encoded by the DNA sequence.

Included within a transcription unit are three critical regions: a promoter, an RNA-coding region, and a terminator (Figure 13.6). The **promoter** is a DNA sequence that the transcription apparatus

recognizes and binds. The promoter indicates which of the two DNA strands is to be read as the template and the direction of transcription. It also determines the transcription start site, the first nucleotide that will be transcribed into RNA. In many transcription units, the promoter is located next to the transcription start site but is not itself transcribed.



13.6 A transcription unit includes a promoter, an RNA-coding region, and a terminator.

Description

The top strand running from 5 prime end to 3 prime end is labeled as nontemplate strand, and the bottom strand running from 3 prime to 5 prime strand is labeled as template strand. From left to right, promoter is labeled on the left side of both strands. To its right, transcription start site begins, along with the R N A-coding region which ends at the Transcription termination site after a terminator sequence on the right side of the strands. A double headed arrow shows upstream on the left and downstream on the right. A transcription termination site is marked. A R N A transcript is formed below that from 5 prime to 3 prime.

The second critical region of the transcription unit is the **RNA-coding region**, a sequence of DNA nucleotides that is copied into an

RNA molecule. The third component of the transcription unit is the **terminator**, a sequence of nucleotides that signals where transcription is to end. Terminators are usually part of the RNA-coding sequence; transcription stops only after the terminator has been copied into RNA.

Molecular biologists often use the terms *upstream* and *downstream* to refer to the direction of transcription and the locations of nucleotide sequences surrounding the RNA-coding region. The transcription apparatus is said to move downstream during transcription: it binds to the promoter (which is usually upstream of the transcription start site) and moves toward the terminator (which is downstream of the start site).

When DNA sequences are written out, often the sequence of only one of the two strands is listed. Molecular biologists typically write the sequence of the nontemplate strand because it will be the same as the sequence of the RNA transcribed from the template strand (with the exception that U in RNA replaces T in DNA). By convention, the sequence of the nontemplate strand is written with the $5'$ end on the left and the $3'$ end on the right. The first nucleotide transcribed (the transcription start site) is numbered +1; nucleotides downstream of the start site are assigned positive numbers, and nucleotides upstream of the start site are assigned negative numbers. So, nucleotide +34 would be 34 nucleotides downstream of the start site, whereas nucleotide −75 would be 75 nucleotides upstream of the start site. There is no nucleotide numbered 0.

CONCEPTS

A transcription unit is a stretch of DNA that encodes an RNA molecule and the sequences necessary for its proper transcription. Each transcription unit includes a promoter, an RNA-coding region, and a terminator.

✔ CONCEPT CHECK 3

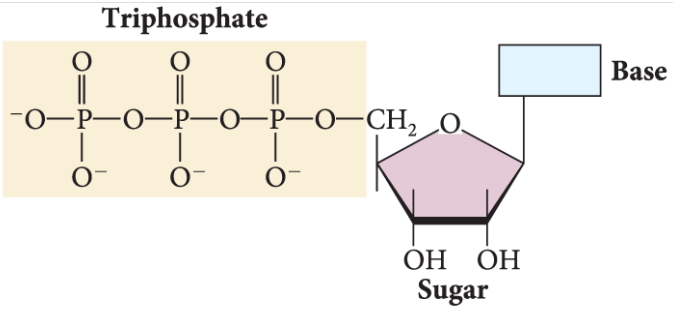
Which of the following phrases does *not* describe a function of the promoter?

a. Serves as the sequence to which the transcription apparatus binds
b. Determines the first nucleotide that is transcribed into RNA
c. Determines which DNA strand is the template
d. Signals where transcription ends

The Substrate for Transcription

RNA is synthesized from **ribonucleoside triphosphates (rNTPs)**, each consisting of a ribose sugar and a base (a nucleoside) attached to three phosphate groups (**Figure 13.7**). In RNA synthesis, nucleotides are added one at a time to the 3′-OH group of the growing RNA molecule. Two phosphate groups are cleaved from the incoming ribonucleoside triphosphate; the remaining phosphate group participates in a phosphodiester bond that connects the nucleotide to the growing RNA molecule. The overall chemical reaction for the addition of each nucleotide is

$$RNA_n + rNTP \rightarrow RNA_{n+1} + PP_i$$



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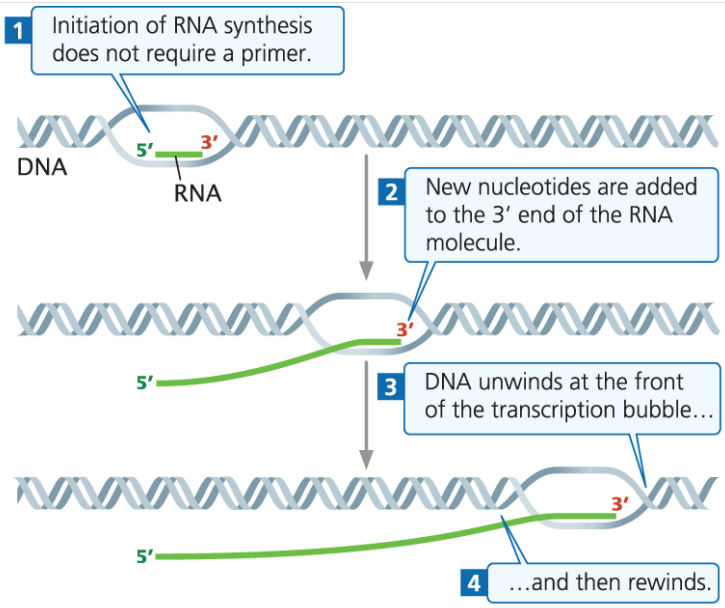
13.7 Ribonucleoside triphosphates are substrates used in RNA synthesis.

Description
A triphosphate chain is bonded to the 5 prime carbon atom of the ribose sugar. A rectangular shaped base is bonded to the 1 prime carbon atom.

where PP_i represents pyrophosphate. Nucleotides are always added to the 3′ end of the RNA molecule, and the direction of transcription is therefore 5′→3′ (Figure 13.8), the same as the direction of DNA synthesis during replication. Thus, the newly synthesized RNA is complementary and antiparallel to the template strand. Unlike DNA synthesis, RNA synthesis does not require a primer.



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13.8 In transcription, nucleotides are always added to the 3′ end of the RNA molecule.

Description

The illustration shows a double helix D N A strand, at the top, with a replication bubble toward the left. A newly synthesized R N A strand runs from 5 prime to 3 prime and is present above the bottom D N A strand. A caption reads, initiation of R N A synthesis does not require a primer. The next step is shown below. As the replication bubble moves to the right, the newly synthesized R N A strand elongates and the 5 prime end extends outside the bubble. A caption reads, new nucleotides are added to the 3 prime end of the R N A molecule. The next step is shown below. As the replication bubble moves further to the right, the newly synthesized R N A strand elongates even more. A caption reads, D N A unwinds at the front of the transcription bubble and then rewinds.

CONCEPTS

RNA is synthesized from ribonucleoside triphosphates. Transcription is 5′→3′: each new nucleotide is joined to the 3′-OH group of the last nucleotide added to the growing RNA

molecule.

The Transcription Apparatus

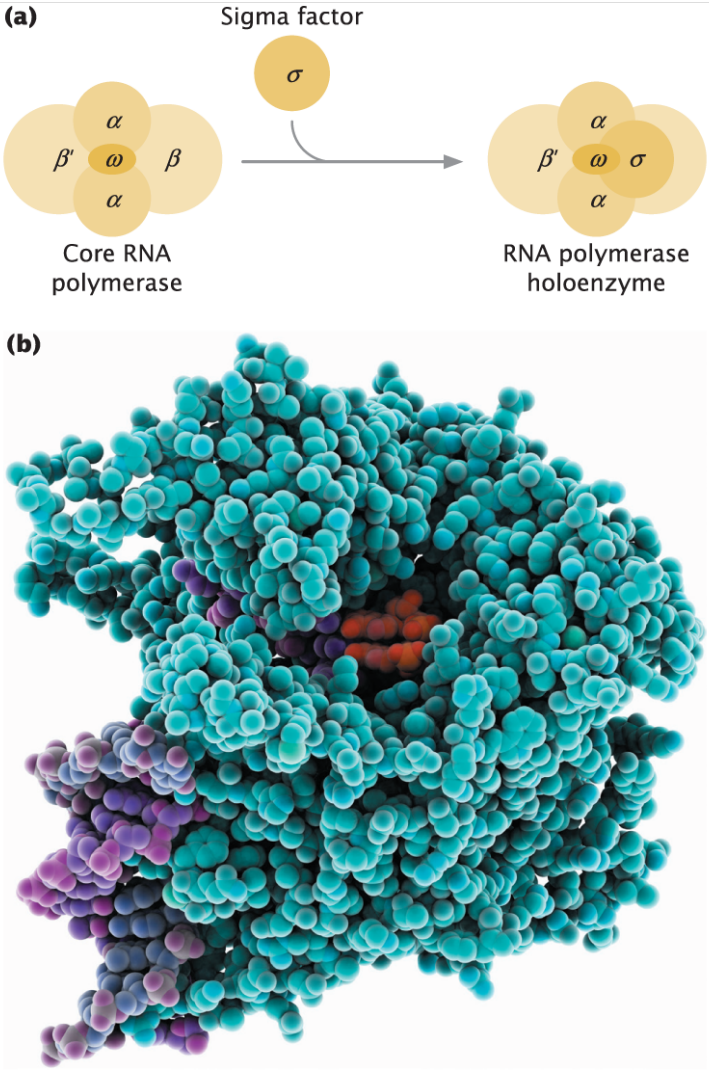
As we have seen, DNA replication requires a number of different enzymes and proteins. Although transcription might initially appear to be quite different because a single enzyme— **RNA polymerase**—carries out all the required steps, the two processes on closer inspection are actually similar. The action of RNA polymerase is enhanced by a number of accessory proteins that join and leave the polymerase at different stages of the process. Each accessory protein is responsible for providing or regulating a special function. Thus, transcription, like replication, requires an array of proteins.

BACTERIAL RNA POLYMERASE

Bacterial cells typically possess only one type of RNA polymerase, which catalyzes the synthesis of all classes of bacterial RNA: mRNA, tRNA, and rRNA. Bacterial RNA polymerase is a large multimeric enzyme (meaning that it consists of several polypeptide chains).

At the heart of most bacterial RNA polymerases are five subunits (individual polypeptide chains) that make up the **core enzyme**: two copies of a subunit called alpha (α) and single copies of subunits

beta (β), beta prime (β'), and omega (ω) (**Figure 13.9**). The ω subunit is not essential for transcription, but it helps stabilize the enzyme. The core enzyme catalyzes the elongation of the RNA molecule by the addition of RNA nucleotides. Other functional subunits join and leave the core enzyme at particular stages of the transcription process. The **sigma (σ) factor** controls the binding of RNA polymerase to the promoter. Without sigma, RNA polymerase initiates transcription at a random point along the DNA. After sigma has associated with the core enzyme (forming a **holoenzyme**), RNA polymerase binds stably only to the promoter and initiates transcription at the proper start site. Sigma is required only for promoter binding and initiation; after a few RNA nucleotides have been joined together, sigma usually detaches from the core enzyme. Many bacteria have multiple types of sigma factors; each type initiates the binding of RNA polymerase to a particular set of promoters.



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Part b: Laguna Design/Science Source.

13.9 In bacterial RNA polymerase, the core enzyme consists of five subunits: two copies of alpha (*α*), a single copy of beta (*β*), a single copy of beta prime (*β′*), and a single copy of omega (*ω*). The core enzyme catalyzes the elongation of the RNA molecule by the addition of RNA nucleotides. (a) The sigma factor (σ) joins the core to form the holoenzyme, which is capable of binding to a promoter and initiating transcription. (b) The molecular model shows RNA polymerase (blue), binding DNA (purple), and synthesizing mRNA (red).

Description

The illustration a at the top shows core R N A polymerase has two copies of alpha, a single copy of beta, a single copy of beta prime , and a single copy of omega. A sigma factor enters the process and attaches to the core R N A polymerase. The core R N A polymerase with sigma factor is collectively labeled R N A polymerase holoenzyme. The molecular model b below shows a larger R N A polymerase represented by large number of blue tiny round structures, binds to the smaller D N A represented by purple tiny round structures, and synthesizes m R N A represented by tiny red round structures present at the center of the model.

Rifamycins are a group of antibiotics that kill bacterial cells by inhibiting RNA polymerase. These antibiotics are widely used to treat tuberculosis, a disease that kills almost 2 million people worldwide each year. The structures of bacterial and eukaryotic RNA polymerases are sufficiently different that rifamycins can inhibit bacterial RNA polymerases without interfering with eukaryotic RNA polymerases. Recent research has demonstrated that several rifamycins work by binding to the part of the bacterial RNA polymerase that clamps onto DNA and then jamming the RNA polymerase, thus preventing RNA polymerase from interacting with the promoter on the DNA.

EUKARYOTIC RNA POLYMERASES

Most eukaryotic cells possess three distinct types of RNA polymerase, each of which is responsible for transcribing a different class of RNA: **RNA polymerase I** transcribes rRNA; **RNA polymerase II** transcribes pre-mRNAs, snoRNAs, some miRNAs, and some snRNAs; and **RNA polymerase III** transcribes other small RNA

molecules—specifically tRNAs, small rRNAs, some miRNAs, and some snRNAs (Table 13.3). RNA polymerases I, II, and III are found in all eukaryotes. Two additional RNA polymerases, **RNA polymerase IV** transcribes siRNAs that silence transposons and **RNA polymerase V** transcribes siRNAs that play a role in DNA methylation and chromatin structure.

TABLE 13.3 Eukaryotic RNA polymerases

Type	Present in	Transcribes
RNA polymerase I	All eukaryotes	Large rRNAs
RNA polymerase II	All eukaryotes	Pre-mRNAs, snoRNAs, some miRNAs, some snRNAs
RNA polymerase III	All eukaryotes	tRNAs, small rRNAs, some miRNAs, some snRNAs
RNA polymerase IV	Plants	siRNAs that silence transposons
RNA polymerase	Plants	siRNAs that affect chromatin

The importance of RNA polymerase is illustrated by α-amanitin, a deadly compound produced by death cap mushrooms. α-Amanitin is a potent inhibitor of RNA polymerase II; it binds to RNA polymerase and jams the moving parts of the enzyme, interfering with its ability to move along the DNA template. In the presence of α-amanitin, RNA synthesis slows from its normal rate of several thousand nucleotides per minute to just a few nucleotides per minute. The results are catastrophic, leading to cell death. The liver, where the toxin accumulates, is irreparably damaged and stops functioning. In severe cases, it causes death.

All eukaryotic polymerases are large multimeric enzymes, typically consisting of more than a dozen subunits. Some subunits are common to all RNA polymerases, whereas others are limited to one of the polymerases. As in bacterial cells, a number of accessory proteins bind to the core enzyme and affect its function.

CONCEPTS

Bacterial cells possess a single type of RNA polymerase, consisting of a core enzyme and other subunits that participate in various stages of transcription. Eukaryotic cells possess several distinct types of RNA polymerase that transcribe different kinds of RNA molecules.

✔ CONCEPT CHECK 4

What is the function of the sigma factor?

13.3 Bacterial Transcription Consists of Initiation, Elongation, and Termination

Now that we've considered some of the major components of transcription, we're ready to take a detailed look at the process. Transcription can be conveniently divided into three stages:

1. Initiation, in which the transcription apparatus assembles on the promoter and begins the synthesis of RNA
2. Elongation, in which DNA is threaded through RNA polymerase and the polymerase unwinds the DNA and adds new nucleotides, one at a time, to the 3′ end of the growing RNA strand
3. Termination, the recognition of the end of the transcription unit and the separation of the RNA molecule from the DNA template

We first examine each of these steps in bacterial cells, in which the process is best understood; then we consider eukaryotic and archaeal transcription.

Initiation

Initiation comprises all the steps necessary to begin RNA synthesis, including (1) promoter recognition, (2) formation of a transcription

bubble, (3) creation of the first bonds between rNTPs, and (4) escape of the transcription apparatus from the promoter.

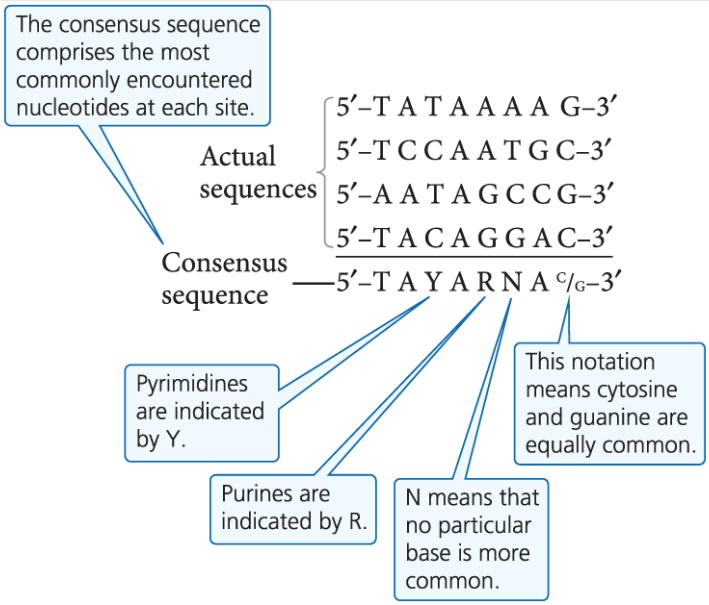
Transcription initiation requires that the transcription apparatus recognize and bind to the promoter. At this step, the selectivity of transcription is enforced: the binding of RNA polymerase to the promoter determines which parts of the DNA template are to be transcribed, and how often. Different genes are transcribed with different frequencies, and promoter binding is important in determining the frequency of transcription for a particular gene. Promoters also have different affinities for RNA polymerase. Even within a single promoter, affinity for RNA polymerase can vary with the passage of time, depending on the promoter's interaction with RNA polymerase and a number of other factors.

BACTERIAL PROMOTERS

Essential information for the transcription apparatus—where it will start transcribing, which strand is to be read, and in what direction the RNA polymerase will move—is embedded in the nucleotide sequence of the promoter. In bacterial cells, promoters are usually adjacent to the RNA-coding region.

An examination of many promoters in *E. coli* and other bacteria reveals a general feature: although most of the nucleotides at most sites vary among these promoters, short stretches of nucleotides are

common to many. Furthermore, the locations of these nucleotides relative to the transcription start site are similar in most promoters. These short stretches of common nucleotides are called consensus sequences. A **consensus sequence** is the set of the most commonly encountered nucleotides among sequences that possess considerable similarity, or *consensus* (Figure 13.10). The presence of consensus in a set of nucleotides usually implies that the sequence is associated with an important function. ❯ **TRY** PROBLEM 21



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13.10 A consensus sequence consists of the most commonly encountered nucleotides at each site in a group of related sequences.

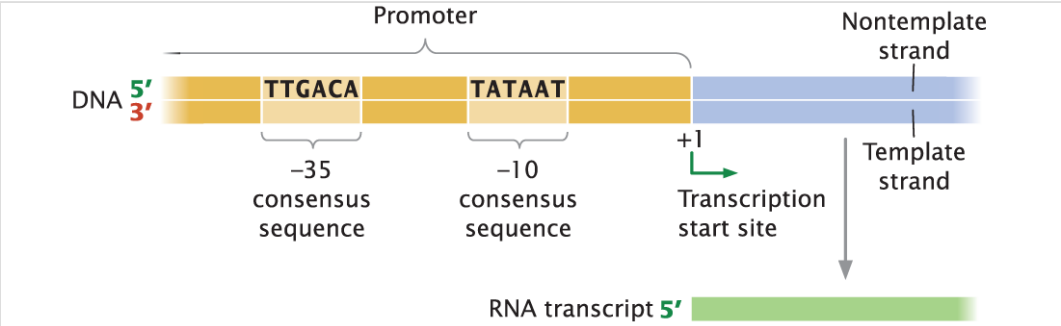
Description
The illustration shows 4 actual sequences and a consensus sequence. The first actual sequence, at the top, from 5 prime to 3 prime is as follows: T, A, T, A, A, A, A, G. The second actual sequence below from 5 prime to 3 prime is as follows: T, C, C, A, A, T, G, C. The third actual sequence below from 5 prime to 3 prime is as follows: A, A, T, A, G, C, C,

G. The fourth actual sequence below from 5 prime to 3 prime is as follows: T, A, C, A, G, G, A, C. The consensus sequence below is as follows: T, A, Y, A, R, N, A, C slash G. A caption reads, the consensus sequence comprises the most commonly encountered nucleotides at each site. Pyrimidines are indicated by the letter Y. Purines are indicated by R. N means that no particular base is more common. This notation C over G means cytosine and guanine are equally common.

The most commonly encountered consensus sequence, found in almost all bacterial promoters, is centered about 10 bp upstream of the start site. Called the **–10 consensus sequence**, or sometimes the Pribnow box, this consensus sequence,

$$5'-\text{TATAAT}-3'$$
$$3'-\text{ATATTA}-5'$$

is often written simply as TATAAT (**Figure 13.11**). Remember that TATAAT is just the *consensus* sequence—representing the most commonly encountered nucleotides at each of these sites (see Figure 13.10). In most prokaryotic promoters, the actual sequence is not TATAAT.



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13.11 In bacterial promoters, consensus sequences are found upstream of the start site, approximately at positions − 10 and − 35.

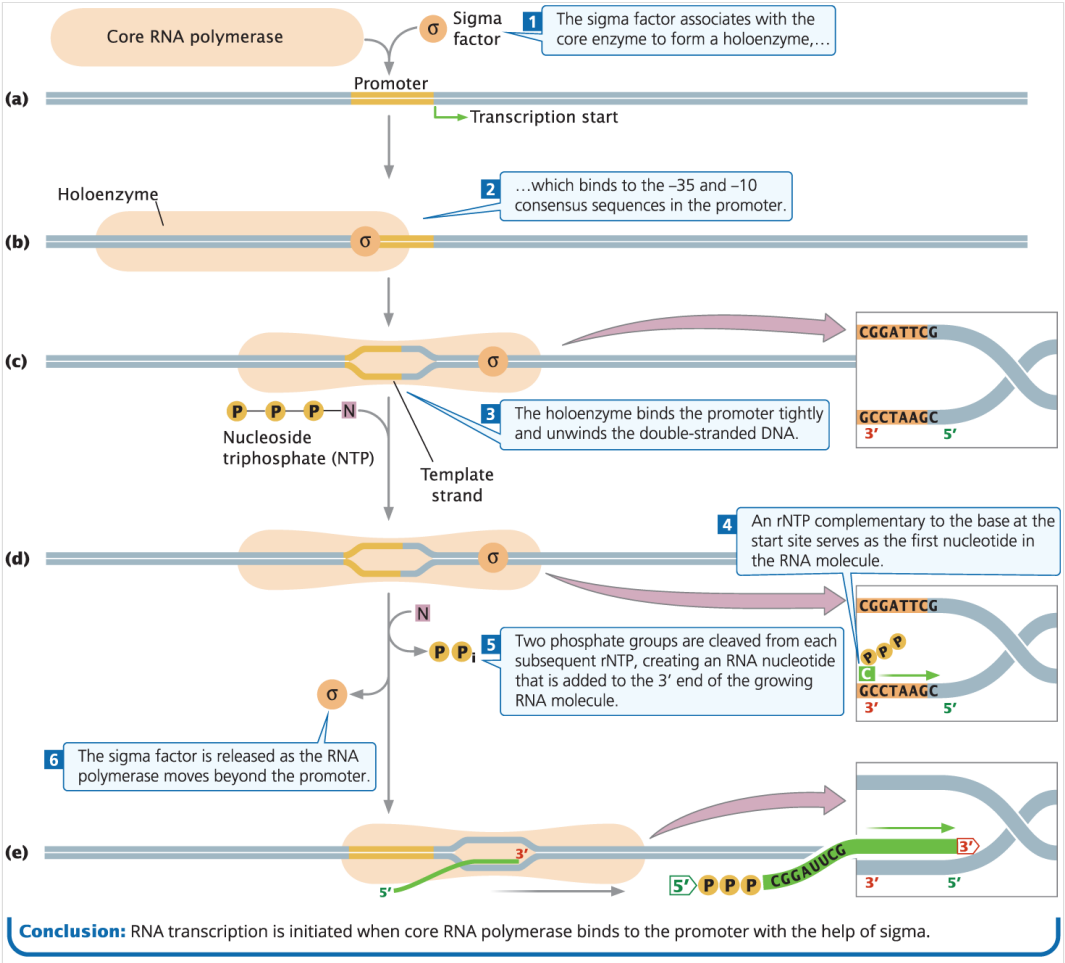
Description
The illustration shows a double stranded D N A molecule with a promoter on the left. The top D N A strand runs from 5 prime to 3 prime while the bottom strand runs from 3 prime to 5 prime. Two consensus sequences are marked at negative 35, on the left, and negative 10 sequences, in the center. Their sequences are as follows respectively: T, T, G, A, C, A and T, A, T, A, A, T. At the positive 1 position, on the right, of the bottom D N A strand, an arrow is drawn from left to the right, indicating the transcription start site. The R N A is being transcribed from the template strand which is the bottom D N A strand. The top D N A strand is labeled as non-template strand. The R N A transcript formed below starts from the 5 prime end.

Another consensus sequence common to most bacterial promoters is TTGACA, which lies approximately 35 nucleotides upstream of the start site and is termed the **− 35 consensus sequence** (see Figure 13.11). The nucleotides on either side of the − 10 and − 35 consensus sequences and those between them vary greatly from promoter to promoter, suggesting that these nucleotides are not very important in promoter recognition.

The function of these consensus sequences in bacterial promoters has been studied by inducing mutations at various positions within the consensus sequences and observing the effect of the changes on transcription. These studies reveal that most base substitutions within the − 10 and − 35 consensus sequences reduce the rate of transcription; these substitutions are termed *down mutations* because they slow down the rate of transcription. Occasionally, a

particular change in a consensus sequence increases the rate of transcription; such a change is called an *up mutation*.

The sigma factor, mentioned earlier, associates with the core RNA polymerase enzyme (**Figure 13.12a**) to form a holoenzyme, which binds to the − 35 and − 10 consensus sequences in the DNA promoter (**Figure 13.12b**). Although it binds only the nucleotides of the consensus sequences, the enzyme extends from −50 to +20 when bound to the promoter. The holoenzyme initially binds weakly to the promoter but then undergoes a change in structure that allows it to bind more tightly and unwind the double-stranded DNA (**Figure 13.12c**). Unwinding begins within the −10 consensus sequence and extends downstream for about 14 nucleotides, including the start site (from nucleotides to − 12 to + 2).



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13.12 Transcription in bacteria is carried out by RNA polymerase, which must bind to the sigma factor to initiate transcription.

Description

Part a at the top shows a double stranded D N A that has a promoter in around the middle of the molecule. The end of the promoter is marked as transcription start. A sigma factor and a core R N A polymerase interact with the promoter. A caption reads, the sigma factor associates with the core to form a holoenzyme. Part b below shows a holoenzyme that binds to the consensus sequences. A caption reads, which binds to the negative 35 and negative 10 consensus sequences in the promoter. Part c below shows the formation of a replication bubble as the holoenzyme moves to the right. To the right of the replication bubble is the sigma factor. A magnified image shows the following sequence on the top strand: C, G, G, A, T, T, C, G and the following sequence on the bottom strand: G, C, C, T, A, A, G, C. A caption reads, the holoenzyme binds the promoter tightly and unwinds the

double-stranded D N A. A nucleoside triphosphate (N T P) molecule enters the process. Part d below shows two replication bubbles form and a newly synthesized R N A strand and extends out from the sigma factor at the end of the second replication bubble. A magnified image shows the first nucleotide C added just over the first nucleotide G of the bottom strand. The nucleotide has three phosphate molecules over it. Two phosphate groups (P and P subscript i) are cleaved from each subsequent r N T P. A caption reads, an r N T P complementary to the base at the start site serves as the first nucleotide in the R N A molecule. Two phosphate groups are cleaved from each subsequent r N T P, creating an R N A nucleotide that is added to the 3 prime end of the growing R N A molecule. The sigma factor is also released. A caption reads, the sigma factor is released as the R N A polymerase moves beyond the promoter. Part e below shows the R N A transcript elongates as the polymerase moves towards the right. A magnified image, on the bottom right, shows the newly synthesized R N A transcript. Three phosphates are attached at the 5 prime end, and the sequence is as follows: C, G, G, A, U, U, C, G.

Some bacterial promoters contain a third consensus sequence that also takes part in the initiation of transcription. Called the **upstream element**, this sequence contains a number of A–T pairs and is found at about − 40 to − 60. A number of proteins may bind to sequences in and near the promoter; some stimulate the rate of transcription and others repress it. We will consider these proteins, which regulate gene expression, in Chapter 16. ❯ **TRY PROBLEM 24**

THINK-PAIR-SHARE Question 2

CONCEPTS

A promoter is a DNA sequence that is adjacent to a gene and required for transcription. Promoters contain short consensus sequences that are important in the initiation of transcription.

✔ CONCEPT CHECK 5

What binds to the −10 consensus sequence found in most bacterial promoters?

a. The holoenzyme (core enzyme + sigma factor)
b. The sigma factor alone
c. The core enzyme alone
d. mRNA

INITIAL RNA SYNTHESIS

Once the holoenzyme has bound to the promoter, RNA polymerase is positioned over the transcription start site (at position +1) and has unwound the DNA to produce a single-stranded template. The orientation and spacing of the consensus sequences on a DNA strand determine which strand will be the template for transcription and thereby determine the direction of transcription.

The position of the start site is determined not by the sequences located there but by the locations of the consensus sequences, which position RNA polymerase so that the enzyme's active site is aligned for the initiation of transcription at +1. If the consensus sequences are artificially moved upstream or downstream, the location of the starting point of transcription correspondingly changes.

To begin the synthesis of an RNA molecule, RNA polymerase pairs the base at the start site on the DNA template strand with its

complementary base on an rNTP (Figure 13.12d). No primer is required to initiate the synthesis of the 5′ end of the RNA molecule. Two of the three phosphate groups are cleaved from each rNTP as the nucleotide is added to the 3′ end of a growing RNA molecule. However, because the 5′ end of the first rNTP does not take part in the formation of a phosphodiester bond, all three of its phosphate groups remain. An RNA molecule therefore possesses, at least initially, three phosphate groups at its 5′ end (Figure 13.12e).

Often in the course of initiation, RNA polymerase repeatedly generates and releases short transcripts, from 2 to 6 nucleotides in length, while still bound to the promoter. This process, termed **abortive initiation**, occurs in both prokaryotes and eukaryotes. After several abortive initiation attempts, the polymerase synthesizes an RNA molecule from 9 to 12 nucleotides in length, which allows the polymerase to transition to the elongation stage.

Elongation

At the end of initiation, RNA polymerase undergoes a change in its conformation (shape) and thereafter is no longer able to bind to the consensus sequences in the promoter. This change allows the polymerase to escape from the promoter and begin transcribing downstream. The sigma factor is usually released after initiation, although some RNA polymerases may retain sigma throughout elongation.

As it moves downstream along the template, RNA polymerase progressively unwinds the DNA at the leading (downstream) edge of the transcription bubble, joining nucleotides to the growing RNA molecule according to the sequence of the template, and rewinds the DNA at the trailing (upstream) edge of the bubble. In bacterial cells at 37°C, about 40 nucleotides are added per second. This rate of RNA synthesis is much lower than that of DNA synthesis, which is 1000 to 2000 nucleotides per second in bacterial cells.

THE TRANSCRIPTION BUBBLE

Transcription takes place within a short stretch of about 18 nucleotides of unwound DNA—the transcription bubble. Within this region, RNA is continuously synthesized. About 8 to 10 nucleotides of newly synthesized RNA are paired with nucleotides on the DNA template at any one time. As the transcription apparatus moves down the template, it generates positive supercoiling ahead of the transcription bubble and negative supercoiling behind it. Topoisomerase enzymes probably relieve the stress associated with the unwinding and rewinding of DNA in transcription, as they do in DNA replication.

TRANSCRIPTIONAL PAUSING

A number of features of RNA or DNA, such as secondary structures, specific sequences, or the presence of nucleosomes, cause RNA

polymerase to pause during the elongation stage of transcription. Such pauses are often caused by backtracking—when the RNA polymerase slides backward along the DNA template strand. Backtracking disengages the 3′-OH group of the RNA molecule from the active site of RNA polymerase and temporarily halts further RNA synthesis. Cells use several mechanisms to minimize backtracking, including proteins that cleave the backtracked RNA in the active site, generating a new 3′-OH group to which new nucleotides can then be added. In bacterial cells, translation of mRNA by ribosomes closely follows transcription (see Chapter 15), and the presence of ribosomes moving along the mRNA in a 5′→3′ direction prevents backtracking of the RNA polymerase at the 3′ end of the mRNA. Backtracking is important in transcriptional proofreading, as we will see shortly.

Transitory pauses in transcription are important in the coordination of transcription and translation in bacteria (see the discussion of attenuation in Chapter 16), as well as in the coordination of RNA processing in eukaryotes. Pausing also affects the rate of RNA synthesis. Sometimes a pause may be stabilized by sequences in the DNA that ultimately lead to the termination of transcription.

ACCURACY OF TRANSCRIPTION

Although RNA polymerase is quite accurate in incorporating nucleotides into the growing RNA chain, errors do occasionally arise. Research has demonstrated that RNA polymerase is capable of a

type of proofreading in the course of transcription. When RNA polymerase incorporates a nucleotide that does not match the DNA template, it backtracks and cleaves the last two nucleotides (including the misincorporated nucleotide) from the growing RNA chain. RNA polymerase then proceeds forward, transcribing the DNA template again.

CONCEPTS

Transcription is initiated at the transcription start site, which, in bacterial cells, is determined by the binding of RNA polymerase to consensus sequences of the promoter. No primer is required. Transcription takes place within a transcription bubble. DNA is unwound ahead of the bubble and rewound behind it. There are frequent pauses in the process of transcription.

Termination

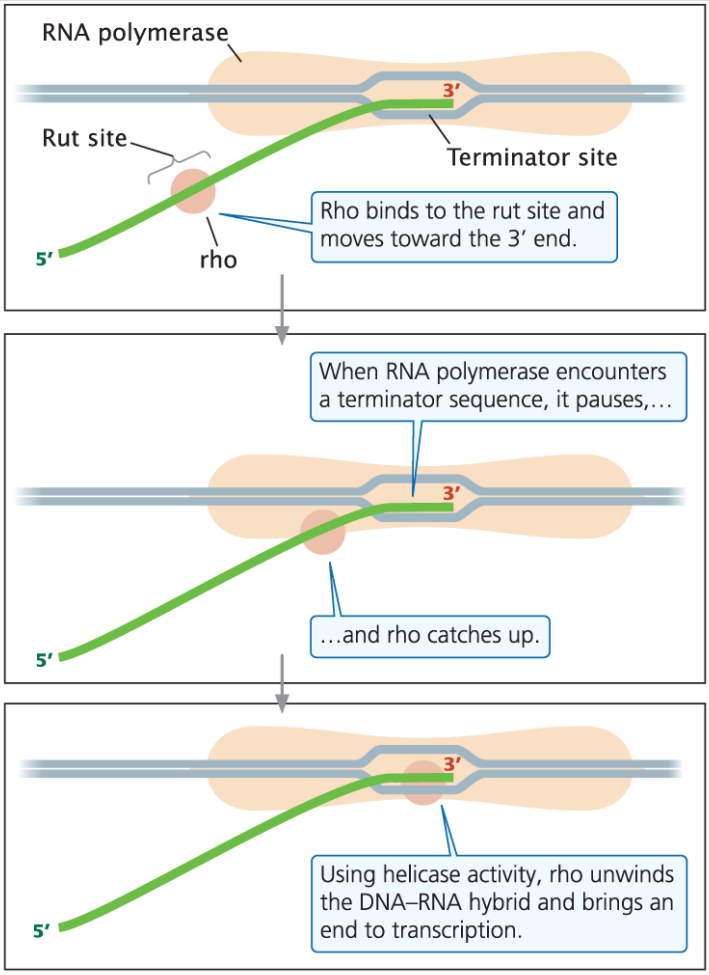
RNA polymerase adds nucleotides to the 3′ end of the growing RNA molecule until it transcribes a terminator. Most terminators are found upstream of the site at which termination actually takes place. Transcription therefore does not suddenly stop when polymerase reaches a terminator, like a car stopping at a stop sign. Rather, transcription stops after the terminator has been transcribed, like a car that stops only after running over a speed bump. At the terminator, several overlapping events are needed to bring an end to transcription: RNA polymerase must stop synthesizing RNA, the

newly made RNA molecule must be released from RNA polymerase, the RNA molecule must dissociate fully from the DNA, and RNA polymerase must detach from the DNA template.

Bacterial cells possess two major types of terminators. **Rho-dependent terminators** are able to cause the termination of transcription only in the presence of an ancillary protein called the **rho factor (ρ)**. **Rho-independent terminators** (also known as intrinsic terminators) are able to cause the end of transcription in the absence of the rho factor.

RHO-DEPENDENT TERMINATORS

Rho-dependent terminators have two features. The first is the DNA sequence of the terminator itself; this sequence causes the RNA polymerase to pause. The second feature is a DNA sequence upstream of the terminator that encodes a stretch of RNA that is usually rich in cytosine nucleotides and devoid of any secondary structures. This RNA sequence, called the rho utilization (rut) site, serves as a binding site for the rho factor. Once rho binds to the RNA, it moves toward its $3'$ end, following the RNA polymerase (**Figure 13.13**). When RNA polymerase encounters the terminator, it pauses, allowing rho to catch up. The rho factor has helicase activity, which it uses to unwind the DNA–RNA hybrid in the transcription bubble, bringing transcription to an end.



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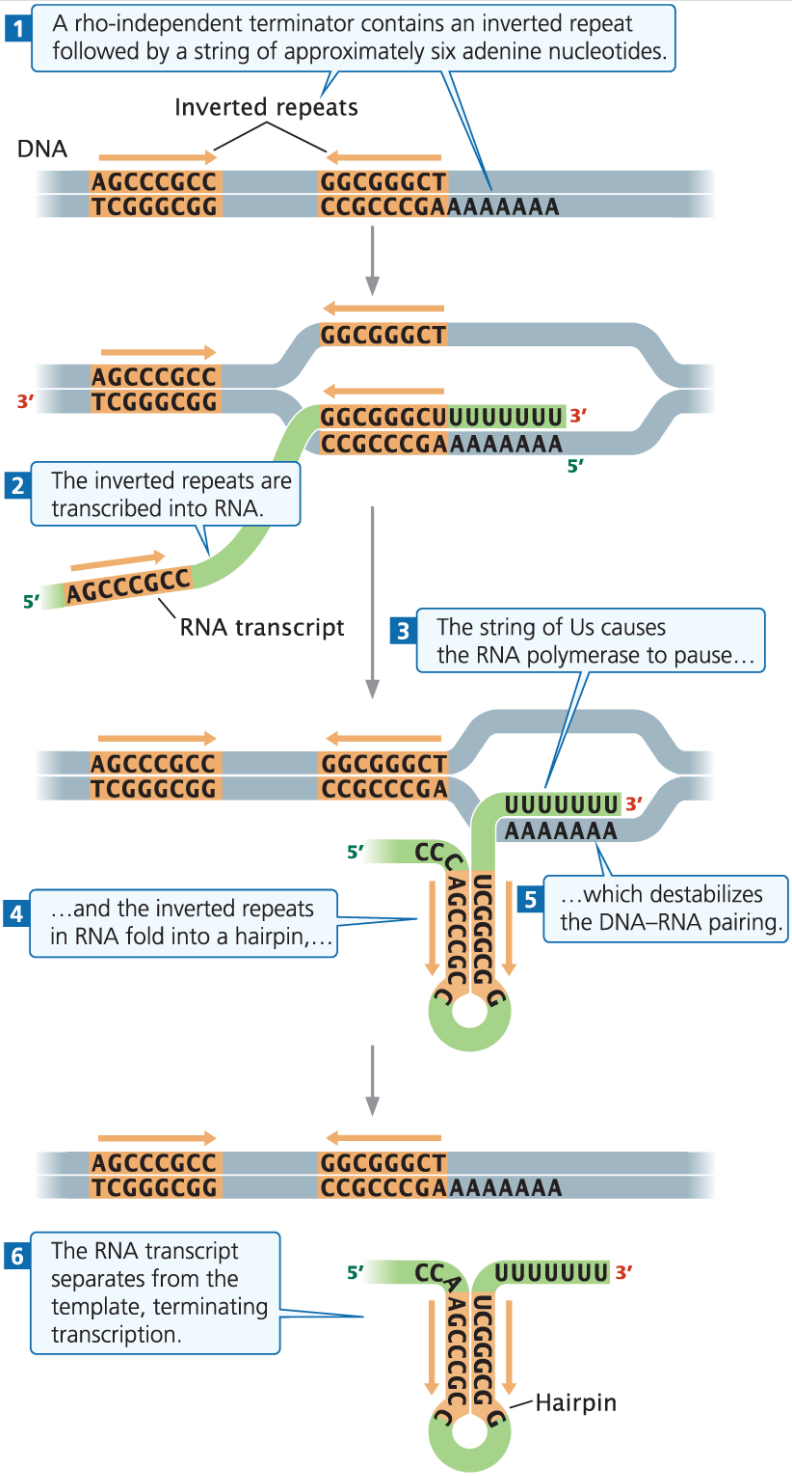
13.13 The termination of transcription in some bacterial genes requires the presence of the rho factor.

Description

The illustration shows R N A polymerase bound to the double stranded D N A around the termination site. The R N A sequence has the round shaped protein r h o attached to it on a site labeled as rut site. A terminator site is marked on the bottom strand. A caption reads, R h o binds to the rut site and moves toward the 3 prime end. In the next step below, the R N A polymerase encounters the terminator sequence. A caption reads, when R N A polymerase encounters a terminator sequence, it pauses and r h o catches up. In the next step below, the r h o protein moves along the R N A sequence toward the end where the termination site is located. A caption reads, using helicase activity, r h o unwinds the D N A-R N A hybrid and brings an end to transcription.

RHO-INDEPENDENT TERMINATORS

Rho-independent terminators, which make up about 50% of all terminators in prokaryotes, have two common features. First, they contain inverted repeats, which are sequences of nucleotides on the same strand that are inverted and complementary. When these inverted repeats are transcribed into RNA and bind to each other, a hairpin forms (Figure 13.14). Second, in rho-independent terminators, a string of seven to nine adenine nucleotides follows the inverted repeat in the template DNA. The transcription of these adenines produces a string of uracil nucleotides after the hairpin in the transcribed RNA.



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13.14 Rho-independent termination in bacteria is a multistep process.

Description
The illustration shows two D N A strands containing 2 closely spaced inverted repeat sequences, and a R N A strand transcribed from this section of the D N A. The first inverted repeat has an arrow over it pointing right and the second inverted repeat has an arrow pointing left. On the template D N A sequence, right after the second inverted repeat is a string of A's. A caption reads, a r h o-independent terminator contains an inverted repeat follow by a string of approximately six adenine nucleotides. A newly synthesized R N A is formed below with the inverted repeats transcribed into it. A caption reads, the inverted repeats are transcribed into R N A. The U's at the end of the R N A transcript cause the polymerase to pause. A caption reads, the string of U's causes the R N A polymerase to pause and the inverted repeats in R N A fold into a hairpin, which stabilizes the D N A-R N A pairing. In the next step below, the hair pin loop separates from the template. A caption reads, the R N A transcript separates from the template, terminating transcription. A caption reads, Conclusion: Transcription terminates when inverted repeats form a hairpin followed by a string of uracils.

The string of uracils in the RNA molecule causes the RNA polymerase to pause, allowing time for the hairpin structure to form. Evidence suggests that the formation of the hairpin destabilizes the DNA–RNA pairing, causing the RNA molecule to separate from its DNA template. Separation may be facilitated by the adenine–uracil base pairings, which are relatively weak compared with other types of base pairings. When the RNA transcript has separated from the template, RNA synthesis can no longer continue (see Figure 13.14). › **TRY PROBLEM 29**

POLYCISTRONIC mRNA

In bacteria, a group of genes is often transcribed into a single RNA molecule; such a molecule is termed **polycistronic mRNA**. Thus, polycistronic mRNA is produced when a single terminator is present at the end of a group of several genes that are transcribed together, instead of each gene having its own terminator. Polycistronic mRNA does occur in some eukaryotes, such as *Caenorhabditis elegans*, but it is uncommon in eukaryotes.

You can view the process of transcription, including initiation, elongation, and termination, in (A) **Animation 13.1**. The animation shows how the different parts of the transcription unit interact to bring about the complete synthesis of an RNA molecule.

THINK-PAIR-SHARE Question 3

CONCEPTS

Transcription ends after RNA polymerase transcribes a terminator. Bacterial cells possess two types of terminators: a rho-independent terminator, which can end transcription by itself; and a rho-dependent terminator, which can end transcription only in the presence of the rho factor.

✔ CONCEPT CHECK 6

What characteristics are most commonly found in rho-independent terminators?

CONNECTING CONCEPTS CONNECT

The Basic Rules of Transcription

Before we examine the process of eukaryotic transcription, let's summarize some of the general principles that we've observed in our exploration of bacterial transcription.

1. Transcription is a selective process; only certain parts of the DNA are transcribed at any one time.
2. RNA is transcribed from a single strand of DNA. Within a gene, only one of the two DNA strands—the template strand—is usually copied into RNA.
3. Ribonucleoside triphosphates are used as the substrates in RNA synthesis. Two phosphate groups are cleaved from a ribonucleoside triphosphate, and the resulting nucleotide is joined to the $3'$-OH group of the growing RNA strand.
4. The transcribed RNA molecule is antiparallel and complementary to the DNA template strand. Transcription is always in the $5' \rightarrow 3'$ direction, meaning that the RNA molecule grows at the $3'$ end.
5. Transcription depends on RNA polymerase, a large multimeric enzyme. RNA polymerase consists of a core enzyme, which is capable of synthesizing RNA, and other subunits that may join transiently to perform additional functions.
6. A sigma factor enables the core enzyme of RNA polymerase to bind to a promoter and initiate transcription.
7. Promoters contain short sequences crucial to the binding of RNA polymerase to DNA; these consensus sequences are interspersed with nucleotides that play no known role in transcription.
8. RNA polymerase binds to DNA at a promoter, begins transcribing at the start site of the gene, and ends transcription after a terminator has been transcribed.
9. Topoisomerase enzymes remove supercoiling that develops ahead of and behind the transcription bubble as the DNA is unwound and rewound during transcription.

13.4 Eukaryotic Transcription Is Similar to Bacterial Transcription but Has Some Important Differences

Transcription in eukaryotes is similar to bacterial transcription in that it includes initiation, elongation, and termination, and the basic principles of transcription already outlined apply to eukaryotic transcription. However, there are some important differences between bacterial and eukaryotic transcription. Eukaryotic cells possess three different RNA polymerases, each of which transcribes a different class of RNA and recognizes a different type of promoter. Thus, a generic promoter cannot be described for eukaryotic cells; rather, a promoter's description depends on whether the promoter is recognized by RNA polymerase I, II, or III. Another difference is in the nature of promoter recognition and initiation. Many accessory proteins take part in the binding of eukaryotic RNA polymerases to DNA templates, and the different types of promoters require different proteins.

Transcription and Nucleosome Structure

Transcription requires that sequences on DNA be accessible to RNA polymerase and other proteins. In eukaryotic cells, however, DNA is complexed with histone proteins in highly compressed chromatin (see Figure 11.4). How can the proteins necessary for transcription gain access to eukaryotic DNA when it is complexed with histones?

The answer to this question is that chromatin structure is modified before transcription so that the DNA is in a more open configuration and is more accessible to the transcription machinery. Several types of proteins have roles in chromatin modification, as noted in Chapter 11. Acetyltransferases, for example, add acetyl groups to amino acids at the ends of the histone proteins, which destabilize nucleosome structure and make the DNA more accessible. Other types of histone modification, such as the addition of methyl groups, can also affect chromatin packing. In addition, chromatin-remodeling proteins may bind to the chromatin and displace nucleosomes from promoters and other regions important for transcription. We will take a closer look at the changes in chromatin structure associated with gene expression in Chapter 17.

CONCEPTS

The initiation of transcription in eukaryotes requires modification of chromatin structure so that DNA is accessible to the transcription machinery.

Promoters

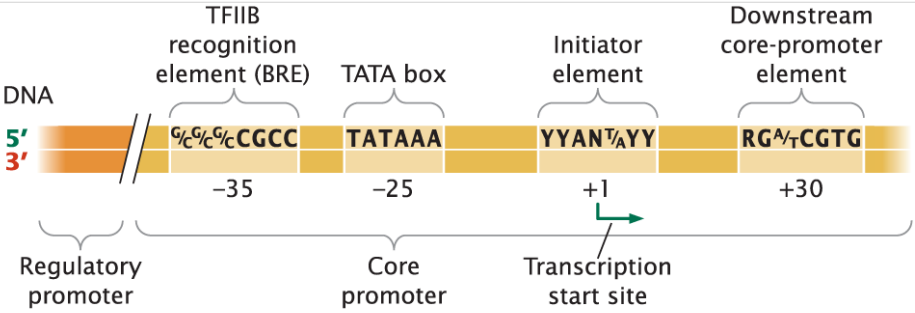
A significant difference between bacterial and eukaryotic transcription is the existence of three different eukaryotic RNA polymerases, which recognize different types of promoters. In bacterial cells, the holoenzyme (the RNA polymerase core enzyme plus the sigma factor) recognizes and binds directly to sequences in the promoter. In eukaryotic cells, promoter recognition is carried out by accessory proteins that bind to the promoter and then recruit a specific RNA polymerase (I, II, or III).

One class of accessory proteins comprises **transcription factors**, which bind to DNA sequences and affect levels of transcription. One group of these, called **general transcription factors**, combine with RNA polymerase to form the **basal transcription apparatus**—a group of proteins that assembles near the transcription start site and is sufficient to initiate minimal levels of transcription. Other transcription factors bind to other DNA sequences and bring about higher levels of transcription by stimulating the assembly of the basal transcription apparatus at the start site.

Here we focus our attention on promoters recognized by RNA polymerase II, which transcribes the genes that encode proteins. A promoter for a gene transcribed by RNA polymerase II typically consists of two primary parts: the core promoter and the regulatory promoter.

CORE PROMOTER

The **core promoter** is located immediately upstream of the gene (Figure 13.15) and is the site to which the basal transcription apparatus binds. The core promoter typically includes one or more consensus sequences. One of the most common of these sequences is the **TATA box**, which has the consensus sequence TATAAA and is located − 25 to − 30 bp upstream of the start site. Additional consensus sequences that may be found in the core promoters of genes transcribed by RNA polymerase II are shown in Figure 13.15. These consensus sequences are recognized by certain transcription factors, which bind to them and serve as a platform for the assembly of the basal transcription apparatus.



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13.15 The promoters of genes transcribed by RNA polymerase II consist of two primary parts: a core promoter and a regulatory promoter. Both parts typically contain consensus sequences, but not all the consensus sequences shown here are found in all promoters.

Description

The regulatory promoter, on the extremne left, is upstream the start site and the core promoter region starts after regulatory promoter. The transcription start site is marked

below initiator element at positive 1 position. The 4 core promoters from left to right are as follows:

T F 2 B recognition element (B R E) has sequence G over C, G slash C, G slash C, C, G, C, C. The T F 2 B recognition element is at the negative 35 position.

T A T A box has sequence T, A, T, A, A, A. The T A T A box is at negative 25 position.

Initiator element has sequence Y, Y, A, N, T slash A, Y, Y. The initiator element is at positive 1 position.

Downstream core-promoter element has sequence R, G, A slash T, C, G, T, G. The downstream core-promoter element is at positive 30 position.

REGULATORY PROMOTER

The **regulatory promoter** is located immediately upstream of the core promoter (see Figure 13.15). A variety of different consensus sequences can be found in regulatory promoters, and they can be mixed and matched in different combinations. Transcription factors bind to these sequences and, either directly or indirectly, make contact with the basal transcription apparatus and affect the rate at which transcription is initiated. Transcription factors may also regulate transcription by binding to more distant sequences called **enhancers**. The DNA between an enhancer and the promoter loops out so that transcription factors bound to the enhancer can interact with the basal transcription apparatus at the core promoter. Enhancers will be discussed in more detail in Chapter 17.

POLYMERASE I AND III PROMOTERS

RNA polymerase I and RNA polymerase III each recognize promoters that are distinct from those recognized by RNA polymerase II. For example, promoters for small rRNA and tRNA genes, transcribed by RNA polymerase III, contain **internal promoters** that are downstream of the start site and are transcribed into the RNA.

CONCEPTS

In eukaryotes, general transcription factors and RNA polymerase assemble on promoters to form the basal transcription apparatus, which binds to DNA near the transcription start site and is necessary for transcription to take place at minimal levels. Other transcription factors bind to other consensus sequences in promoters and enhancers and affect the rate of transcription.

✔ CONCEPT CHECK 7

What is the difference between the core promoter and the regulatory promoter?

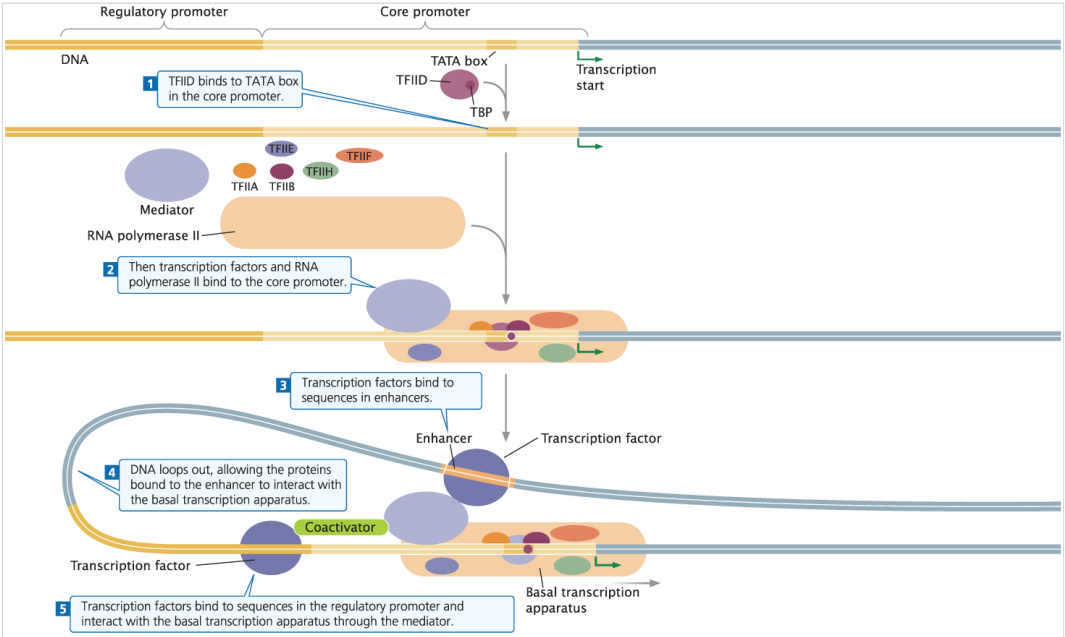
a. Only the core promoter has consensus sequences.
b. The regulatory promoter is farther upstream of the gene.
c. Transcription factors bind only to the core promoter and not to the regulatory promoter.
d. Both b and c.

Initiation

Transcription in eukaryotes is initiated through the assembly of the transcription machinery on the promoter. This machinery consists of RNA polymerase II and a series of transcription factors that form

a giant complex consisting of 50 or more polypeptides. Assembly of the transcription machinery begins when regulatory proteins bind DNA near the promoter and modify the chromatin structure so that transcription can take place. These and other regulatory proteins then recruit the basal transcription apparatus to the core promoter.

The basal transcription apparatus consists of RNA polymerase II, a series of general transcription factors, and a complex of proteins known as the mediator (Figure 13.16). The general transcription factors include TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH, in which *TFII* stands for "*t*ranscription *f*actor for RNA polymerase *II*" and the final letter designates the individual factor.



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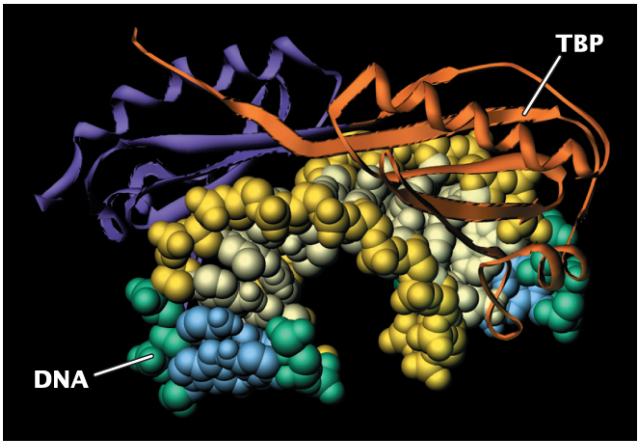
13.16 Transcription is initiated at RNA polymerase II promoters. Transcription is initiated by the binding of the TFIID transcription factor to the TATA box, followed by the binding of a preassembled holoenzyme containing general transcription

factors, RNA polymerase II, and the mediator. TBP stands for “TATA-binding protein.”

Description
The illustration shows a double D N A strand at the top. The D N A has the following from left to right: the regulatory promoter, core promoter with a T A T A box, and a transcription start site. A T F 2 D molecule with T B P binds at the T A T A box. A caption reads, T F 2 D binds to the T A T A box in the core promoter. A mediator, transcription factors T F 2 A, T F 2 B, T F 2 E, T F 2 H, T F 2 F, and R N A polymerase 2 enter the process. A caption reads, then transcription factors and R N A polymerase 2 bind to the core promoter. In the next step below, the D N A loops out. A transcription factor attaches at the end of regulatory promoter (co-activator) and a transcription factor attaches at the enhancer on the looped out D N A strand. A co-activator extends between mediator and transcription factor. The basal transcription apparatus moves towards the right. A caption reads, transcription factors bind to sequences in enhancers. D N A loops out, allowing the proteins bound to the enhancer to interact with the basal transcription apparatus. Transcription factors bind to sequences in the regulatory promoter and interact with the basal transcription apparatus through the mediator.

RNA polymerase II and the general transcription factors assemble at the core promoter, forming a pre-initiation complex. Recall that in bacteria, the sigma factor recognizes and binds to the promoter sequence. In eukaryotes, the function of sigma is replaced by that of the general transcription factors. A first step in initiation is the binding of TFIID to the TATA box on the core promoter. TFIID consists of at least nine polypeptides. One of those polypeptides is the **TATA-binding protein (TBP)**, which recognizes and binds to the TATA box. The TATA-binding protein binds to the minor groove of the DNA double helix and straddles the DNA like a molecular saddle (Figure 13.17), bending the DNA and partly unwinding it. Other

general transcription factors bind to additional consensus sequences in the core promoter and to RNA polymerase II, and position the polymerase over the transcription start site.



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13.17 The TATA-binding protein (TBP) binds to the minor groove of DNA, straddling the double helix of DNA like a saddle.

Description
The molecular model shows ribbon structure of T B P which is straddling the D N A molecule. The D N A molecule is represented in an almost semi-circular shape through a space-filling model.

After the RNA polymerase and general transcription factors have assembled on the core promoter, conformational changes take place in both the DNA and the polymerase. These changes cause 11 to 15 bp of DNA surrounding the transcription start site to unwind, producing the single-stranded DNA that will serve as a template for transcription.

The DNA template strand is positioned within the active site of the RNA polymerase, creating a structure called the open complex. After the open complex has formed, the synthesis of RNA begins as phosphate groups are cleaved off rNTPs and nucleotides are joined together to form an RNA molecule. As in bacterial transcription, the RNA polymerase may generate and release several short RNA molecules in abortive initiation attempts before it initiates the synthesis of a full-length RNA molecule. › **TRY** PROBLEM 35

CONCEPTS

Transcription in eukaryotes is initiated when the basal transcription apparatus—consisting of RNA polymerase, general transcription factors, and a mediator—assembles on the core promoter and becomes an open complex.

✔ CONCEPT CHECK 8

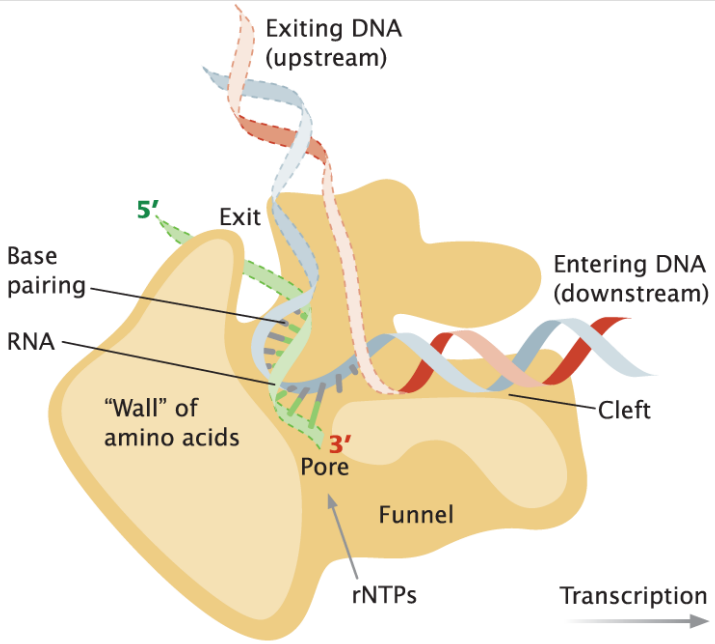
What is the role of TFIID in transcription initiation?

Elongation

After about 30 bp of RNA have been synthesized, the RNA polymerase moves downstream of the promoter and begins the elongation stage of transcription. Many of the general transcription factors are left behind at the promoter, where they can serve to

quickly reinitiate transcription with another RNA polymerase enzyme.

The molecular structure of eukaryotic RNA polymerase II and how it functions during elongation were revealed through the work of Roger Kornberg and his colleagues, for which Kornberg was awarded a Nobel Prize in chemistry in 2006. The RNA polymerase maintains a transcription bubble during elongation, in which about eight nucleotides of the growing RNA molecule remain paired with the DNA template strand. The DNA double helix enters a cleft in the polymerase and is gripped by jawlike extensions of the enzyme (**Figure 13.18**). The two strands of the DNA are unwound, and nucleotides that are complementary to the template strand are added to the growing 3′ end of the RNA molecule. As it funnels through the polymerase, the DNA–RNA hybrid hits a wall of amino acids and bends at almost a right angle; this bend positions the end of the DNA–RNA hybrid at the active site of the polymerase, where new nucleotides are added to the 3′ end of the growing RNA molecule. The newly synthesized RNA is separated from the DNA and runs through another cleft in the enzyme before exiting from the polymerase.



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13.18 The structure of RNA polymerase II is a source of insight into its function.
The DNA double helix enters RNA polymerase II through a cleft in the enzyme and unwinds. The DNA–RNA duplex is bent at a right angle, which positions the 3′ end of the RNA at the active site of the enzyme. At the active site, new nucleotides are added to the 3′ end of the growing RNA molecule.

Description

The illustration shows different regions of the R N A polymerase 2 molecule. The left half is labeled “wall” of amino acids. The bottom is labeled as funnel and in the middle of the polymerase is a pore. At the pore, the 3 prime end of the R N A is marked and r N T P’s enter the funnel. At the top of the R N A polymerase is an opening is present from where the 5 prime end of the R N A strand exits. On the right side of the polymerase is a region labeled as cleft, where downstream double stranded D N A enters the polymerase. At the top, upstream D N A is exiting after it gets unwounded in the middle of the molecule. The D N A strand serves as a template for R N A synthesis. A newly synthesized R N A strand leaves from the top, parallel to the exiting D N A. Transcription direction is marked towards the right.

Termination

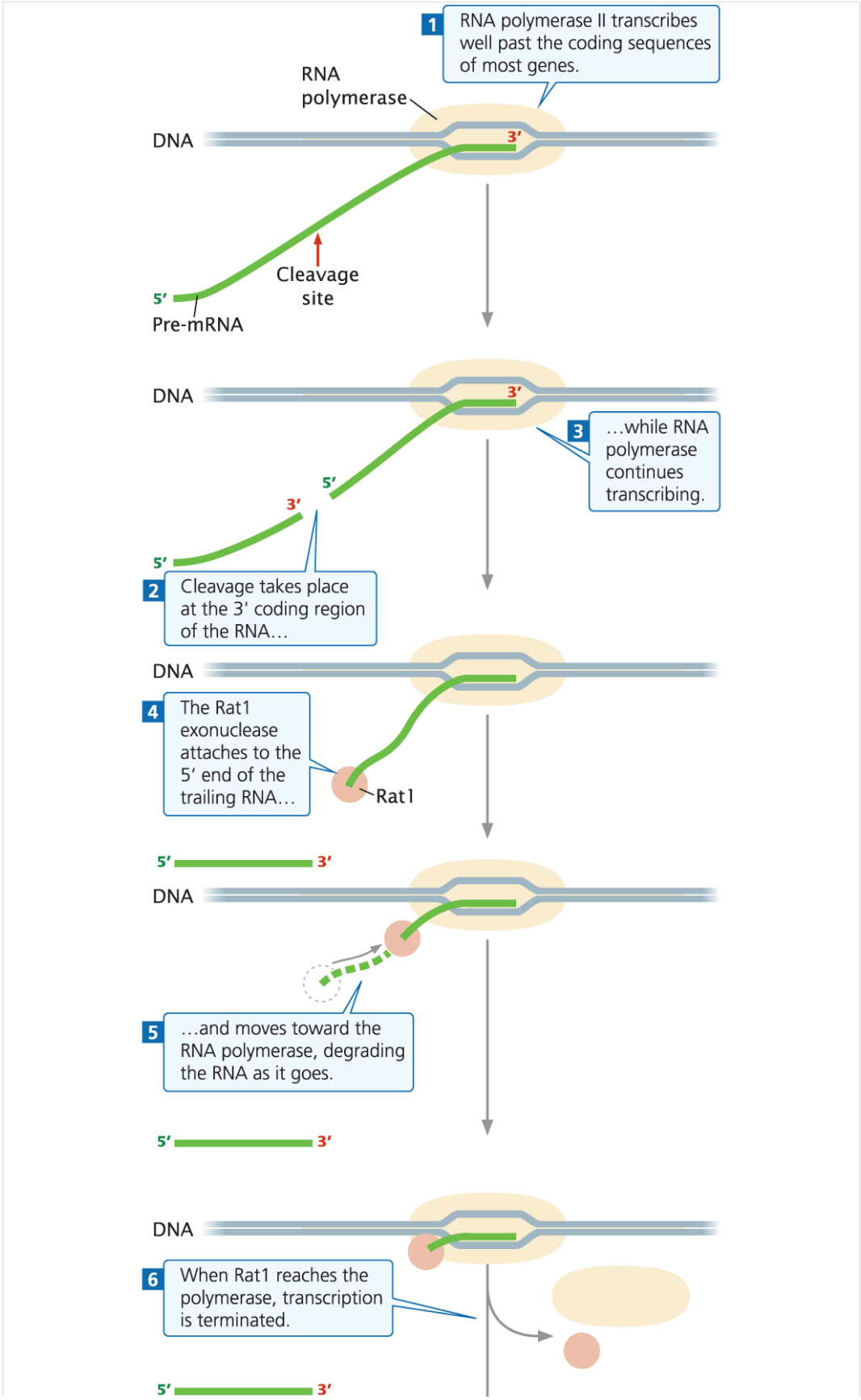
The three eukaryotic RNA polymerases use different mechanisms for termination. RNA polymerase I requires a termination factor similar to the rho factor used in terminating the transcription of some bacterial genes. Unlike rho, which binds to the newly transcribed RNA molecule, the termination factor for RNA polymerase I binds to a DNA sequence downstream of the terminator.

RNA polymerase III ends transcription after transcribing a terminator sequence that produces a string of uracil nucleotides in the RNA molecule. Recent research demonstrates that secondary structures, such as hairpins and stems, often occur upstream of the string of uracils and are necessary for termination. Thus, RNA polymerase III uses a termination mechanism similar to rho-independent termination in bacteria.

The termination of transcription by RNA polymerase II does not occur at specific sequences. Instead, RNA polymerase II often continues to synthesize RNA hundreds or even thousands of nucleotides past the coding sequence necessary to produce the mRNA. As we will see in Chapter 14, the pre-mRNA is cleaved at a specific site, designated by a consensus sequence, while transcription is still taking place at the $3'$ end of the molecule. Cleavage cuts the pre-mRNA into two pieces: the mRNA that will eventually encode the protein and another piece of RNA that has its $5'$ end trailing out of the RNA polymerase (**Figure 13.19**). An enzyme

(called Rat1 in yeast) attaches to the 5′ end of this RNA and moves toward the 3′ end, where the RNA polymerase continues the transcription of RNA. Rat1 is a 5′→3′ exonuclease—an enzyme capable of degrading RNA in the 5′→3′ direction. Like a guided torpedo, Rat1 homes in on the polymerase, chewing up the RNA as it moves. When Rat1 reaches the transcription machinery, transcription terminates. Note that this mechanism is similar to that of rho-dependent termination in bacteria (see Figure 13.13), except that rho does not degrade the RNA molecule.

THINK-PAIR-SHARE Question 4


1
RNA polymerase II transcribes well past the coding sequences of most genes.
RNA polymerase
DNA
3′
Cleavage site
5′
Pre-mRNA
DNA
3′
3
…while RNA polymerase continues transcribing.
5′
3′
5′
2
Cleavage takes place at the 3' coding region of the RNA…
DNA
4
The Rat1 exonuclease attaches to the 5′ end of the trailing RNA…
Rat1
5′
3′
DNA
5
…and moves toward the RNA polymerase, degrading the RNA as it goes.
5′
3′
DNA
6
When Rat1 reaches the polymerase, transcription is terminated.
5′
3′

13.19 Termination of transcription by RNA polymerase II requires the Rat1 exonuclease. Cleavage of the pre-mRNA produces a $5'$ end to which Rat1 attaches. Rat1 degrades the RNA molecule in the $5' \rightarrow 3'$ direction. When Rat1 reaches the polymerase, transcription is halted.

Description

The illustration shows two D N A strands at the top. A R N A polymerase molecule slides along the lower D N A strand and a replication bubble is formed to initiate R N A synthesis. The pre-m R N A strand formed elongates and its 5 prime end extends out while the 3 prime end remains in the replication bubble. In the middle of this pre-m R N A strand is a cleavage site. A caption reads, R N A polymerase 2 transcribes well past the coding sequences of most genes. In the next step below, cleavage at the cleavage point occurs while the pre-m R N A was still elongating. A caption reads, cleavage is near the 3 prime end of the R N A while R N A polymerase continues transcribing. In the next step below, the attached 5 prime end of the pre-m R N A transcript has a round structure R a t 1 exonuclease attached to it. The cleaved pre-m R N A transcript remains intact. A caption reads, the R a t 1 exonuclease attaches to the 5 prime end of the trailing R N A. In the next step below, the exonuclease moves towards the R N A polymerase as it degrades the R N A simultaneously. The cleaved pre-m R N A transcript remains intact. A caption reads, and moves toward the R N A polymerase, degrading the R N A as it goes. In the next step below, R a t 1 reaches the polymerase, transcription stops. R a t 1 and D N A polymerase 2 are released from the reaction. The cleaved pre-m R N A transcript remains intact throughout. In the end, the D N A strands resume the original shape.

CONCEPTS

The three RNA polymerases found in all eukaryotes use different mechanisms of termination. Transcription by RNA polymerase II is terminated when an exonuclease enzyme attaches to the cleaved $5'$ end of the RNA, moves down the RNA, and reaches the polymerase enzyme.

✔ CONCEPT CHECK 9

How are the processes of RNA polymerase II termination in eukaryotes and rho-dependent termination in bacteria similar, and how are they different?

13.5 Transcription in Archaea Is More Similar to Transcription in Eukaryotes Than to Transcription in Bacteria

Some 2 billion to 3 billion years ago, life diverged into three lines of evolutionary descent: the bacteria (also called eubacteria), the archaea, and the eukaryotes (see Chapter 2). Although bacteria and archaea are superficially similar—both are unicellular and lack a nucleus—the results of studies of their DNA sequences and other biochemical properties indicate that they are as distantly related to each other as they are to eukaryotes. The evolutionary distinctions between archaea, bacteria, and eukaryotes are clear. But did eukaryotes first diverge from an ancestral prokaryote, with a later separation of prokaryotes into bacteria and archaea, or did the archaea and the bacteria split first, with the eukaryotes later evolving from one of these groups?

Studies of transcription in bacteria, archaea, and eukaryotes have yielded important clues to the evolutionary relationships between these organisms. Archaea, like bacteria, have a single RNA polymerase, but this enzyme is most similar to the RNA polymerases of eukaryotes. As discussed earlier, bacterial RNA polymerase consists of 5 subunits, whereas eukaryotic RNA polymerases are much more complex; RNA polymerase II, for

example, is composed of 12 subunits. Archaeal RNA polymerase is similarly complex, with 11 or more subunits. Furthermore, the amino acid sequence of archaeal RNA polymerase is similar to that of eukaryotic RNA polymerase II.

Archaeal promoters contain a consensus sequence similar to the TATA box found in eukaryotic promoters. The archaeal TATA box is found approximately 27 bp upstream of the transcription start site and helps to determine the location of the transcription start site, as it does in eukaryotes. Archaea possess a TATA-binding protein (TBP), which is a critical transcription factor for all three of the eukaryotic polymerases but not for bacterial RNA polymerase. TBP binds the TATA box in archaea with the help of another transcription factor, TFIIB, which is also found in eukaryotes but not in bacteria. However, some other regulators of transcription found in archaea are more similar to those found in bacteria, so transcription in archaea is not entirely eukaryotic in nature. As prokaryotes, archaea lack a nuclear membrane, but many species do produce histone proteins, which help compact the DNA and form nucleosome-like structures.

Thus, transcription, one of the most basic of life processes, has strong similarities in eukaryotes and archaea, suggesting that these two groups are more closely related to each other than either is to bacteria. This conclusion is supported by other data, including those obtained from a comparison of gene sequences.

CONCEPTS

The process of transcription in archaea has many similarities to transcription in eukaryotes.

Chapter 13 Review

CHAPTER SUMMARY

Section 13.1 Learning Objective: Be able to explain how the structure of RNA allows it to participate in a variety of cellular functions.

- Early life used RNA both as the carrier of genetic information and as a biological catalyst.
- RNA is a polymer consisting of nucleotides joined together by phosphodiester bonds. Each RNA nucleotide consists of a ribose sugar, a phosphate group, and a nitrogenous base. In contrast to DNA, RNA contains the base uracil and is usually single stranded, which allows it to form secondary structures.
- Cells possess a number of different classes of RNA. Ribosomal RNA is a component of the ribosome, messenger RNA carries coding instructions for proteins, and transfer RNA helps incorporate amino acids into a polypeptide chain.

Section 13.2 Learning Objective: Be able to describe the major components required for transcription.

- The template for RNA synthesis is single-stranded DNA. In transcription, RNA synthesis is complementary and antiparallel

to the DNA template strand. A transcription unit consists of a promoter, an RNA-coding region, and a terminator.
- The substrates for RNA synthesis are ribonucleoside triphosphates.
- RNA polymerase in bacterial cells consists of a core enzyme, which catalyzes the addition of nucleotides to an RNA molecule and other subunits. The sigma factor controls the binding of the core enzyme to the promoter.
- Eukaryotic cells contain multiple types of RNA polymerases.

Section 13.3 Learning Objective: Be able to outline the process of bacterial transcription.

- The process of transcription consists of three stages: initiation, elongation, and termination.
- Transcription begins at the start site, which is determined by consensus sequences in a promoter. A short stretch of DNA is unwound near the start site, RNA is synthesized from a single template strand of DNA, and the DNA is rewound at the lagging end of the transcription bubble. RNA polymerases are capable of proofreading. There are frequent pauses in the process of transcription.
- RNA synthesis ceases after a terminator sequence has been transcribed. Bacterial cells have two types of terminators: rho-independent terminators and rho-dependent terminators.

Section 13.4 Learning Objective: Be able to compare and contrast transcription in bacteria and eukaryotes.

- The initiation of transcription in eukaryotes requires the modification of chromatin structure. Different types of RNA polymerases in eukaryotes recognize different types of promoters.
- For genes transcribed by RNA polymerase II, general transcription factors, which bind to the core promoter, are part of the basal transcription apparatus. Other transcription factors bind to sequences in regulatory promoters and enhancers and interact with the basal transcription apparatus.
- The three RNA polymerases found in all eukaryotes use different mechanisms of termination.

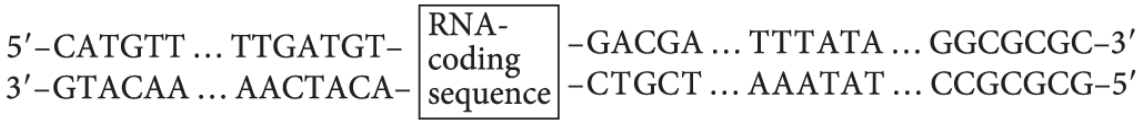
Section 13.5 Learning Objective: Be able to compare transcription in archaea to that in bacteria and eukaryotes.

- Transcription in archaea has many similarities to transcription in eukaryotes.

WORKED PROBLEMS

Problem 1

The accompanying diagram represents a sequence of nucleotides surrounding an RNA-coding sequence.



Description
The top strand reads, 5 prime C A T G T T ellipsis T T G A T G T, R N A coding sequence, G A C G A ellipsis T T T A T A ellipsis g G G C G C G C 3 prime.

The bottom strand reads, 3 prime G T A C A A ellipsis A A C T A C A, R N A coding sequence, C T G C T ellipsis A A A T A T ellipsis C C G C G C G 5 prime.

a. Is the RNA-coding sequence likely to be from a bacterial cell or from a eukaryotic cell? How can you tell?
b. Which DNA strand will serve as the template strand during the transcription of the RNA-coding sequence?

» Solution Strategy

What information is required in your answer to the problem?

a. Whether the sequence is likely to be from a bacterial or a eukaryotic cell, and why.
b. Which strand is the template strand.

What information is provided to solve the problem?

- The nucleotide sequences of both strands of DNA.
- The $5'$ and $3'$ ends of the strands.

For help with this problem, review:

The Template in Section 13.2, Bacterial Promoters in Section 13.3, and Promoters in Section 13.4.

» Solution Steps

a. Bacterial and eukaryotic cells use the same DNA bases (A, T, G, and C), so the bases themselves provide no clue to the origin of the sequence. The RNA-coding sequence must be accompanied by a promoter, and bacterial and eukaryotic cells do differ in the consensus sequences found in their promoters, so we should examine the sequence for the presence of familiar consensus sequences. On the bottom strand to the right of the RNA-coding sequence, we find AAATAT, which, written in the conventional manner (5′ on the left), is 5′–TATAAA–3′. This sequence is the TATA box, which is found in most eukaryotic promoters. However, the sequence is also quite similar to the − 10 consensus sequence (5′–TATAAT–3′) found in bacterial promoters.

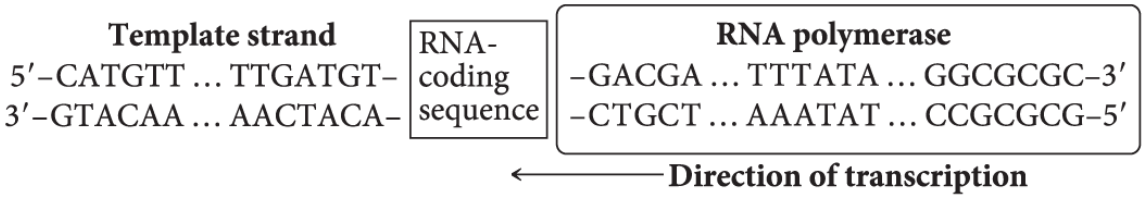
> **Hint:** Review the consensus sequences found in bacterial and eukaryotic promoters in Figures 13.11 and 13.15.

Farther to the right on the bottom strand, we also see 5′–GCGCGCC–3′, which is the TFIIB recognition element (BRE, see **Figure 13.15**) in eukaryotic RNA polymerase II promoters. No similar consensus sequence is found in bacterial promoters, so we can be fairly certain that this sequence is a eukaryotic promoter and an RNA-coding sequence.

b. The TATA box and BRE of RNA polymerase II promoters are upstream of the RNA-coding sequence, so RNA polymerase must bind to these sequences and then proceed downstream,

transcribing the RNA-coding sequence. Thus, RNA polymerase must proceed from right (upstream) to left (downstream). The RNA molecule is always synthesized in the 3′→5′ direction and is antiparallel to the DNA template strand, so the template strand must be read 3′→5′. If the enzyme proceeds from right to left and reads the template in the 3′→5′ direction, the upper strand must be the template, as shown in the accompanying diagram.

Recall: During transcription, the template strand is read 3′→5′.



Description
The illustration shows the following D N A template strand: The upper D N A strand is as follows: 5 prime C, A, T, G, T, T, ellipsis T, T, G, A, T, G, T, then the R N A coding sequence and the beginning of R N A polymerase G, A, C, G, A ellipsis T, T, T, A, T, A ellipsis G, G, C, G, C, G, C 3 prime; end of R N A polymerase. The lower D N A strand is as follows: 3 prime G, T, A, C, A, A ellipsis A, A, C, T, A, C, A, then the R N A coding sequence and the beginning of R N A polymerase C, T, G, C, T ellipsis A, A, A, T, A, T ellipsis C, C, G, C, G, C, G 5 prime; end of R N A polymerase. The direction of transcription is from 5 prime to 3 prime of the lower D N A strand.

Problem 2

Suppose that a consensus sequence in the regulatory promoter of a eukaryotic gene that encodes enzyme A was deleted. Which of the

following effects would result from this deletion? Explain your reasoning.

a. Enzyme A would have a different amino acid sequence.
b. The mRNA for enzyme A would be abnormally short.
c. Enzyme A would be missing some amino acids.
d. The mRNA for enzyme A would be transcribed but not translated.
e. The amount of mRNA transcribed would be affected.

» Solution Strategy

What information is required in your answer to the problem?

The result (*a*, *b*, *c*, *d*, or *e*) that would occur if a consensus sequence in the regulatory promoter were deleted.

What information is provided to solve the problem?

The deleted consensus sequence is in the regulatory promoter.

For help with this problem, review:

Section 13.4.

» Solution Steps

The correct answer is part *e*. The regulatory promoter contains binding sites for transcription factors. These sequences are not part of the RNA-coding sequence for enzyme A, so the mutation would have no effect on the length or the amino acid sequence of the enzyme, eliminating answers *a, b,* and *c*. The regulatory promoter affects transcription, but not translation, so answer d is not correct. The binding of transcription factors to the regulatory promoter affects the amount of transcription that takes place through interactions with the basal transcription apparatus at the core promoter.

IMPORTANT TERMS

ribozyme
ribosomal RNA (rRNA)
messenger RNA (mRNA)
pre-messenger RNA (pre-mRNA)
transfer RNA (tRNA)
small nuclear RNA (snRNA)
small nuclear ribonucleoprotein (snRNP)
small nucleolar RNA (snoRNA)
microRNA (miRNA)
small interfering RNA (siRNA)
Piwi-interacting RNA (piRNA)
long noncoding RNA (lncRNA)
CRISPR RNA (crRNA)
template strand

nontemplate strand
transcription unit
promoter
RNA-coding region
terminator
ribonucleoside triphosphate (rNTP)
RNA polymerase
core enzyme
sigma (σ) factor
holoenzyme
RNA polymerase I
RNA polymerase II
RNA polymerase III
RNA polymerase IV
RNA polymerase V
consensus sequence
−10 consensus sequence (Pribnow box)
−35 consensus sequence
upstream element
abortive initiation
rho-dependent terminator
rho factor (ρ)
rho-independent terminator
polycistronic mRNA
transcription factor
general transcription factor
basal transcription apparatus
core promoter
TATA box

regulatory promoter
enhancer
internal promoter
TATA-binding protein (TBP)

ANSWERS TO CONCEPT CHECKS

1. b
2. The template strand is the DNA strand that is copied into an RNA molecule, whereas the nontemplate strand is not copied.
3. d
4. The sigma factor controls the binding of RNA polymerase to the promoter.
5. a
6. Inverted repeats followed by a string of adenine nucleotides.
7. b
8. TFIID binds to the TATA box and helps to center the RNA polymerase over the transcription start site.
9. Both processes use a protein that binds to the RNA molecule and moves down the RNA toward the RNA polymerase. They differ in that rho does not degrade the RNA, whereas Rat1 does.

Chapter 13 Assessment

COMPREHENSION QUESTIONS

Section 13.1

1. CONNECT Draw an RNA nucleotide and a DNA nucleotide, highlighting the differences. How is the structure of RNA similar to that of DNA? How is it different? (See Chapter 10 for a review of DNA structure.)

2. What are the major classes of cellular RNA?

3. Why is DNA more stable than RNA?

Section 13.2

4. What parts of DNA make up a transcription unit? Draw a typical bacterial transcription unit, and identify its parts.

5. What is the substrate for RNA synthesis? How is this substrate modified and joined together to produce an RNA molecule?

6. Describe the structure of the bacterial RNA polymerase holoenzyme.

7. Give the names of the RNA polymerases found in eukaryotic cells and the types of RNA that they transcribe.

Section 13.3

8. What are the three basic stages of transcription? Describe what happens at each stage.

9. Draw a typical bacterial promoter, and identify any common consensus sequences.

10. What are the two basic types of terminators found in bacterial cells? Describe the structure of each type.

Section 13.4

11. Compare the roles of general transcription factors that bind to the core promoter and other transcription factors that bind to the regulatory promoter and enhancers.

12. How are transcription and replication similar, and how are they different?

13. How is transcription different in bacteria and eukaryotes? How is it similar?

APPLICATION QUESTIONS AND PROBLEMS

Introduction

14. CONNECT Rear-end collisions between replication and transcription (discussed in the introduction to this chapter) are less likely in eukaryotes because the two processes occur at comparable speeds. Bacteria, in contrast, replicate their DNA almost 10 times faster than they carry out transcription. Can you suggest some reasons why bacteria replication is faster than eukaryotic replication?

Section 13.1

15. *An RNA molecule has the following percentages of bases: A = 23%, U = 42%, C = 21%, and G = 14%

    a. Is this RNA single stranded or double stranded? How can you tell?

    b. What would be the percentages of bases in the template strand of the DNA that contains the gene for this RNA?

Section 13.2

16. *The following diagram represents DNA that is part of the RNA-coding sequence of a transcription unit. The bottom strand is the template strand. Give the sequence found on the RNA molecule transcribed from this DNA, and identify the $5'$ and $3'$ ends of the RNA.

5′–ATAGGCGATGCCA–3′

3′–TATCCGCTACGGT–5′ ← Template strand

17. For the RNA molecule shown in **Figure 13.1a**, write out the sequence of bases on the template and nontemplate strands of DNA from which this RNA is transcribed. Label the 5′ and 3′ ends of each strand.

18. The following sequence of nucleotides is found in a single-stranded DNA template:

    ATTGCCAGATCATCCCAATAGAT

    Assume that RNA polymerase proceeds along this template from left to right.

    a. Which end of the DNA template is 5′, and which end is 3′?

    b. Give the sequence and identify the 5′ and 3′ ends of the RNA transcribed from this template.

19. Assume that a mutation occurs in the gene that encodes each of the following RNA polymerases. Match the mutation with its possible effects by placing the correct letter or letters in the blanks below. There may be more than one effect for each mutated polymerase.

A mutation in the gene that codes for	Effects
RNA polymerase I	_____

RNA polymerase II	_____
RNA polymerase III	_____

Possible effects

a. tRNA is not synthesized.

b. Some ribosomal RNA is not synthesized.

c. Ribosomal RNA is not processed.

d. pre-mRNA is not processed.

e. Some mRNA molecules are not degraded.

f. pre-mRNA is not synthesized.

Section 13.3

20. Provide the consensus sequence for the *first three* of the actual sequences shown in **Figure 13.10**.

21. *Write the consensus sequence for the following set of nucleotide sequences.

AGGAGTT
AGCTATT
TGCAATA
ACGAAAA
TCCTAAT
TGCAATT

22. CONNECT List at least five properties that DNA polymerases and RNA polymerases have in common. List at least three differences. See Chapter 12 for information on DNA polymerases.

23. Most RNA molecules have *three* phosphate groups at the 5′ end, but DNA molecules never do. Explain this difference.

24. *Write a hypothetical sequence of bases that might be found in the first 20 nucleotides of a promoter of a bacterial gene. Include both strands of DNA, and identify the 5′ and 3′ ends of both strands. Be sure to include the transcription start site and any consensus sequences found in the promoter.

25. *What would be the most likely effect of a mutation at the following locations in an *E. coli* gene?

    a. −8

    b. −35

    c. −20

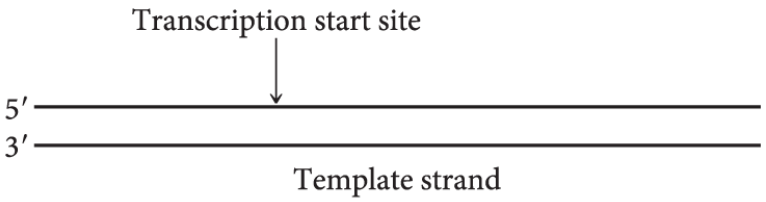
    d. Start site

26. A strain of bacteria possesses a temperature-sensitive mutation in the gene that encodes the sigma factor. The mutant bacteria produce a sigma factor that is unable to bind to RNA polymerase at elevated temperatures. What effect will this

mutation have on the process of transcription when the bacteria are raised at elevated temperatures?

27. On **Figure 13.5**, indicate the locations of the promoters and terminators for genes *a*, *b*, and *c*.

28. The following diagram represents a transcription unit on a DNA molecule.



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Description
D N A double strand is represented by two parallel horizontal lines. The top strand runs from 5 prime and the bottom strand runs from 3 prime. Transcription start site is marked a little before the center of the top strand, using a downward arrow.

a. Assume that this DNA molecule is from a bacterial cell. Draw the approximate locations of the promoter and terminator for this transcription unit.

b. Assume that this DNA molecule is from a eukaryotic cell. Draw the approximate location of an RNA polymerase II promoter.

29. *The following DNA nucleotides are found near the end of a bacterial transcription unit.

3′ – AGCATACAGCAGACCGTTGGTCTGAAAAAAGCATACA – 5′

a. Mark the point at which transcription will terminate.

b. Is this terminator rho-independent or rho-dependent?

c. Draw a diagram of the RNA that will be transcribed from this DNA, including its nucleotide sequence and any secondary structures that form.

30. A strain of bacteria possesses a temperature-sensitive mutation in the gene that encodes the rho subunit. At high temperatures, rho is not functional. When these bacteria are raised at elevated temperatures, which of the following effects would you expect to see? Explain your reasoning for accepting or rejecting each of these five options.

    a. Transcription does not take place.

    b. All RNA molecules are shorter than normal.

    c. All RNA molecules are longer than normal.

    d. Some RNA molecules are longer than normal.

    e. RNA is copied from both DNA strands.

31. Suppose that the string of A nucleotides following the inverted repeat in a rho-independent terminator was deleted but the inverted repeat was left intact. How would this deletion affect termination? What would happen when RNA polymerase reached this region?

32. CONNECT Compare and contrast replication (see chapter 12) and transcription by making a table listing similarities and differences between transcription and replication. List as many as you can think of.

Section 13.4

33. The following diagram represents a transcription unit in a hypothetical DNA molecule.

$$5' \ldots \text{TTGACA} \ldots \text{TATAAT} \ldots 3'$$
$$3' \ldots \text{AACTGT} \ldots \text{ATATTA} \ldots 5'$$

a. On the basis of the information given, is this DNA from a bacterium or from a eukaryotic organism?

b. If this DNA molecule is transcribed, which strand will be the template strand and which will be the nontemplate strand?

c. Where, approximately, will the transcription start site be?

34. Computer programmers, working with molecular geneticists, have developed programs that can identify genes within long stretches of DNA sequence. Imagine that you are working with a programmer on such a project. On the basis of what you know about the process of transcription, what sequences should the program use to identify the beginning and end of a gene?

35. *Through genetic engineering, a geneticist mutates the gene that encodes TBP in cultured human cells. This mutation

destroys the ability of TBP to bind to the TATA box. Predict the effect of this mutation on cells that possess it.

36. CONNECT Elaborate repair mechanisms that prevent permanent mutations in DNA are associated with replication (see Chapter 18), yet no similar repair process is associated with transcription. Can you think of a reason for this difference between replication and transcription? (Hint: Think about the relative effects of a permanent mutation in a DNA molecule and one in an RNA molecule.)

CHALLENGE QUESTIONS

Section 13.3

37. Many genes in both bacteria and eukaryotes contain numerous sequences that can cause pauses in or premature termination of transcription. Nevertheless, the transcription of these genes within a cell normally produces multiple RNA molecules thousands of nucleotides long without pausing or premature termination. However, when a single round of transcription of these genes takes place in a test tube, RNA synthesis is frequently interrupted by pauses and premature terminations, which reduce the rate at which transcription takes place and frequently shorten the lengths of the mRNA molecules produced. Most pauses and premature terminations occur when

RNA polymerase temporarily backtracks (i.e., backs up) for one or two nucleotides along the DNA. Experimental findings have demonstrated that most pauses and premature terminations disappear if several RNA polymerases are simultaneously transcribing the DNA molecule. Propose an explanation for this observation of faster transcription and longer mRNAs when the template DNA is being transcribed by multiple RNA polymerases.

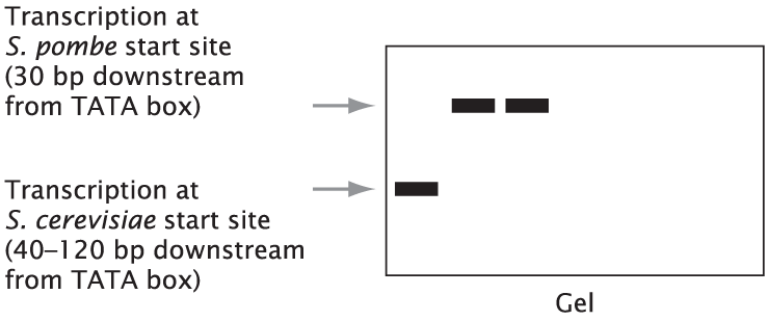
Section 13.4

38. Enhancers are sequences that affect the initiation of the transcription of genes that are hundreds or thousands of nucleotides away. Transcription factors that bind to enhancers usually interact directly with transcription factors at promoters by causing the intervening DNA to loop out. An enhancer of bacteriophage T4 does not function by looping of the DNA (D. R. Herendeen et al. 1992. *Science* 256:1298–1303). Propose some mechanisms other than DNA looping by which this enhancer might affect transcription at a gene thousands of nucleotides away.

39. *The locations of the TATA box in two species of yeast, *Saccharomyces pombe* and *Saccharomyces cerevisiae*, differ dramatically. The TATA box of *S. pombe* is about 30 nucleotides upstream of the transcription start site, similar to the location in most other eukaryotic cells. However, the TATA box of *S. cerevisiae* is 40 to 120 nucleotides upstream of the start site.

To better understand what sets the start site in these organisms, researchers at Stanford University conducted a series of experiments to determine which components of the transcription apparatus of these two species could be interchanged (Y. Li et al. 1994. *Science* 263:805–807). In these experiments, different general transcription factors and RNA polymerases were switched in *S. pombe* and *S. cerevisiae*, and the effects of each switch on the level of RNA synthesis and on the starting point of transcription were observed. The results from one set of experiments are shown in the table below. Components cTFIIB, cTFIIE, cTFIIF, and cTFIIH are general transcription factors from *S. cerevisiae*. Components pTFIIB, pTFIIE, pTFIIF, and pTFIIH are general transcription factors from *S. pombe*. Components cPol II and pPol II are RNA polymerase II from *S. cerevisiae* and *S. pombe*, respectively. The table indicates whether the component was present (+) or missing (–) in each experiment. In the accompanying gel, the presence of a band indicates that RNA was produced, and the position of the band indicates whether it was the length predicted when transcription begins 30 bp downstream of the TATA box or when it begins 40 to 120 bp downstream of the TATA box.

Components	Experiment 1	2	3	4	5	6	7
cTFIIE	+	−	+	+	+	+	−
cTFIIH + cTFIIF	+	−	+	+	+	−	+
cTFIIB + cPol II	+	−	−	−	−	−	−
pPol II	−	+	+	+	−	+	+
pTFIIB	−	+	+	−	+	+	+
pTFIIE + pTFIIH + pTFIIF	−	+	−	−	−	−	−



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Description

The table has 6 rows and 8 columns. The column headers are as follows: Components and Experiment numbers 1 through 7. The row entries are as follows:

Row 1: c T F 2 E; positive, negative, positive, positive, positive, positive, negative.

Row 2: c T F 2 H plus c T F 2 F; positive, negative, positive, positive, positive, negative, positive.

Row 3: c T F 2 B plus c P o l 2; positive, negative, negative, negative, negative, negative, negative.

Row 4: p P o l 2; negative, positive, positive, positive, negative, positive, positive.

Row 5: p T F 2 B; negative, positive, positive, negative, positive, positive, positive.

Row 6: p T F 2 E plus p T F 2 H plus p T F 2 F; negative, positive, negative, negative, negative, negative, negative.

The gel below shows the following information:

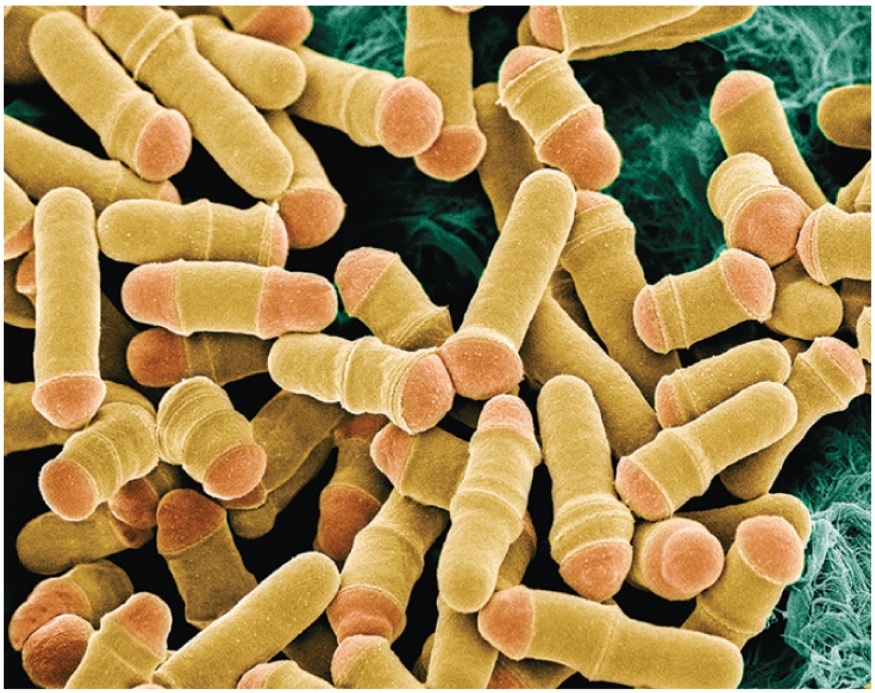
Transcription at S pombe start site (30 b p downstream from T A T A box): blank space, band, band.

Transcription at S cerevisiae start site (40 to 120 b p downstream from T A T A box): band.

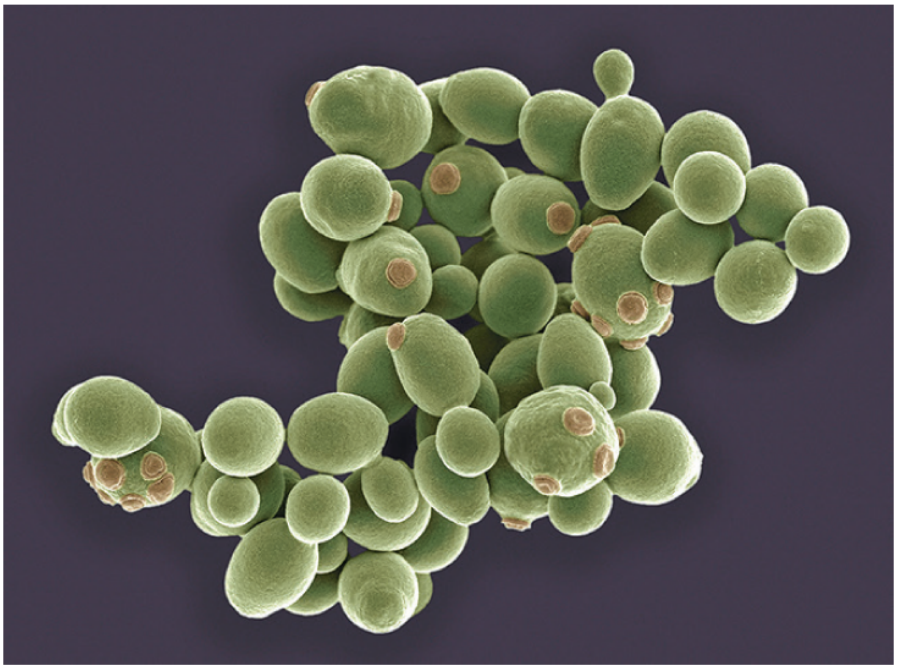
a. What conclusion can you draw from these data about what components determine the start site for transcription?

b. What conclusions can you draw about the interactions of the different components of the transcription apparatus?

c. Propose a mechanism that might have caused the transcription start site in *S. pombe* to be about 30 bp downstream of the TATA box, while the start site for transcription in *S. cerevisiae* is 40 to 120 bp downstream of the TATA box.



Steve Gschmeissner/Science Source.



Steve Gschmeissner/Science Source.

40. DATA ANALYSIS Glenn Croston and his colleagues studied the relation between chromatin structure and transcription activity. In one set of experiments, they measured the level of in vitro transcription of a *Drosophila* gene by RNA polymerase II in the presence of DNA and various combinations of histone proteins (G. E. Croston et al. 1991. *Science* 251:643–649).

    First, they measured the level of transcription of naked DNA, with no associated histone proteins. Then they measured the level of transcription after nucleosome octamers (without H1) were added to the DNA. The addition of the octamers caused the level of transcription to drop by 50%. When both nucleosome octamers and H1 proteins were added to the DNA, transcription was greatly repressed, dropping to less than 1% of that obtained with naked DNA, as shown in the following table.

GAL4-VP16 is a protein that binds to the DNA of certain eukaryotic genes. When GAL4-VP16 is added to DNA, the level of transcription by RNA polymerase II is greatly elevated.

Treatment	Relative amount of transcription
Naked DNA	100
DNA + octamers	50
DNA + octamers + H	+1
DNA + GAL4-VP16	1000
DNA + octamers + GAL4-VP16	1000
DNA + octamers+H1 + GAL4-VP16	1000

Even in the presence of the H1 protein, GAL4-VP16 stimulates high levels of transcription.

Propose a mechanism by which the H1 protein represses transcription and by which GAL4-VP16 overcomes this repression. Explain how your proposed mechanism would produce the results obtained in these experiments.

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

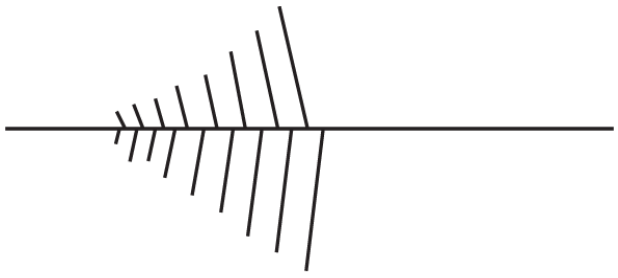
Section 13.2

1. Usually, within a gene, only one of the DNA strands is transcribed into RNA (see Figure 13.5). Why haven't both strands in a DNA molecule evolved to carry genetic information? In other words, why might we not expect both strands to carry genetic information and be transcribed into RNA?

Section 13.3

2. Why do consensus sequences have consensus? In other words, why do copies of consensus sequences in different species tend to have similar nucleotides? Hint: Consensus sequences are usually found in stretches of DNA that have important functions, such as promoters.

3. The following diagram represents one of the Christmas-tree-like structures shown in **Figure 13.3**.



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Description
There are 8 branches above the trunk and 9 branches below the trunk.

On the diagram, identify parts *a* through *i*.

a. DNA molecule
b. 5′ and 3′ ends of the template strand of DNA
c. At least one RNA molecule
d. 5′ and 3′ ends of at least one RNA molecule
e. Direction of movement of the transcription apparatus on the DNA molecule
f. Approximate location of the promoter
g. Possible location of a terminator
h. Upstream and downstream directions
i. Molecules of RNA polymerase (use dots to represent these molecules)

Section 13.4

4. List some of the ways in which eukaryotic transcription is more complex than transcription in bacteria. Propose some possible reasons for the greater complexity of transcription in eukaryotic cells.

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Draw a concept map that illustrates the causes and consequences of collisions between replication and transcription. Include at least the following terms: replication, transcription, collisions, head-on collisions, rear-end collisions, $5'\rightarrow3'$ direction, template DNA, replication stalling, premature termination of transcription, DNA breaks, mutations, co-directional replication and transcription, DNA repair, restarting replication, removing transcription complexes.

Section 13.1

2. Create a concept map that compares and contrasts the structures of DNA and RNA. Include similarities and differences in their structures.

Section 13.2

3. Draw a concept map that summarizes DNA sequences involved in transcription by including the following terms: transcription, DNA template strand, DNA nontemplate strand, promoter, RNA-coding sequence, terminator, transcription start site, RNA molecule, transcription unit.

Section 13.3

4. Outline the process of transcription in bacteria by creating a concept map that includes the following terms: transcription,

ribonucleoside triphosphates, $5' \rightarrow 3'$, RNA polymerase, sigma factor, promoter, holoenzyme, promoter, −10 consensus sequence, −35 consensus sequence, transcription bubble, transcription pausing, transcription initiation, termination, terminator, rho factor, rho-independent terminator, rho-dependent terminator.

Section 13.4

5. Create a concept map that illustrates transcription in eukaryotes by including the following terms: eukaryotic transcription, RNA polymerase I, RNA polymerase II, RNA polymerase III, transcription factors, basal transcription apparatus, regulatory promoter, core promoter, enhancer, TATA box.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com

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CHAPTER 14

RNA Molecules and RNA Processing

Mondadori Portfolio/Getty Images.

Family of Tsar Nicholas Romanov II of Russia. The boy in front is the tsar's son, Alexei, who suffered from hemophilia.

A Royal Disease

On August 12, 1904, Tsar Nicholas Romanov II of Russia wrote in his diary: "A great never-to-be forgotten day when the mercy of God has

visited us so clearly." That day, Alexei, Nicholas's first son and heir to the Russian throne, had been born.

At birth, Alexei was a large and vigorous baby, with yellow curls and blue eyes, but at 6 weeks of age he began spontaneously hemorrhaging from the navel. The bleeding persisted for several days and caused great alarm. As he grew and began to walk, Alexei often stumbled and fell, as all children do. Even his small scrapes bled profusely, and minor bruises led to significant internal bleeding. It soon became clear that Alexei had hemophilia.

Marked by slow clotting and excessive bleeding, hemophilia is caused by a mutation in one of several genes that encode proteins involved in the process of blood clotting. In people with hemophilia, minor injuries can result in life-threatening blood loss, and spontaneous bleeding into joints erodes the bone, with crippling consequences.

Alexei suffered from classic hemophilia, which is an X-linked genetic disorder. He inherited the hemophilia mutation from his mother, Alexandra, who was a carrier. The mutation appears to have originated with Queen Victoria of England (see Figure 6.8). In all, ten of Queen Victoria's male descendants suffered from hemophilia. Six female descendants, including her granddaughter Alexandra (Alexei's mother), were carriers.

During his childhood, Alexei experienced a number of severe bleeding episodes. The royal physicians were often helpless during these crises—they had no treatment that would stop the bleeding. At this time, the Russian Revolution broke out. Bolsheviks captured the tsar and his family and held them captive in the city of Yekaterinburg. On the night of July 16, 1918, a firing squad executed the royal family and their attendants, including Alexei and his four sisters. For many years, the bodies of the tsar's family were lost, but their skeletons were eventually recovered from two graves outside Yekaterinburg. Comparisons of mitochondrial DNA and nuclear DNA from the bones and from descendants of relatives of the family verified that the bones were indeed those of the royal family. Although this analysis determined the identity of the remains, the molecular nature of the royal hemophilia long remained unknown.

In 2009, geneticists analyzed DNA from the bones of the tsar's family to determine the genetic nature of the royal hemophilia. In people with X-linked hemophilia, a mutation commonly occurs in one of two genes on the X chromosome: the gene for blood coagulation factor VIII or the gene for blood coagulation factor IX.

In eukaryotic cells, genes are often interrupted by noncoding sequences. Those parts of a gene that encode the amino acids of a protein are called exons, while the noncoding sequences are called introns. Initially, both exons and introns are transcribed into pre-mRNA, but in a process called RNA splicing, the introns are later cut out and the exons pasted together. Examination of the

sequences of the two clotting factor genes in Alexandra's DNA revealed no mutations in the nucleotides that encode amino acids, but a mutation was present in one of the introns of her gene for factor IX. This mutation altered the splicing of the exons, creating a new stop signal in the coding sequence of the mRNA and producing a truncated, defective clotting factor IX. Both normal and mutant alleles were detected in Alexandra's DNA, as is expected of a heterozygous carrier. DNA from Alexei carried only the mutant allele, which caused his hemophilia.

THINK-PAIR-SHARE

- Classic hemophilia is inherited as an X-linked recessive condition. When a female is heterozygous for an X-linked recessive mutation such as hemophilia, what proportion of her male offspring are expected to have hemophilia? Examine the pedigree of the royal family in Figure 6.8. Calculate the proportion of affected male offspring born to females heterozygous for hemophilia. Carry out a chi-square goodness-of-fit test comparing the numbers of observed and expected male progeny of these females affected by hemophilia. What conclusion can you draw from the chi-square test?
- Hemophilia in the royal family was caused by the presence of a mutation in the gene for factor IX that altered the splicing of the exons and created a new stop signal in the mRNA for factor IX. Explain how a splicing mutation could produce a premature stop signal.

As illustrated by Alexei's hemophilia, RNA processing is critically important for the proper synthesis of proteins. In eukaryotic cells, RNA molecules are often extensively modified after transcription: for genes that encode proteins, a modified nucleotide called the 5′ cap is added to the 5′ end, a tail of adenine nucleotides is added to

the 3′ end, and introns are cut out of the middle. In both prokaryotes and eukaryotes, rRNAs and tRNAs are also modified after transcription.

In Chapter 13, we focused on transcription—the process by which RNA molecules are synthesized. In this chapter, we examine the function and processing of RNA. We begin by taking a careful look at the nature of the gene. We then examine messenger RNA (mRNA), its structure, and how it is modified in eukaryotes after transcription. Then we turn to transfer RNA (tRNA), the adapter molecule that forms the interface between amino acids and mRNA in protein synthesis. We examine ribosomal RNA (rRNA), the structure and organization of rRNA genes, and how rRNAs are processed. Finally, we consider a newly discovered group of very small RNAs that play important roles in numerous biological functions.

As we explore the world of RNA and its role in gene function, we will see evidence of two important characteristics of this nucleic acid. First, RNA is extremely versatile, both structurally and biochemically. It can assume a number of different secondary structures, which provide the basis for its functional diversity. Second, RNA processing and function frequently include interactions between two or more RNA molecules.

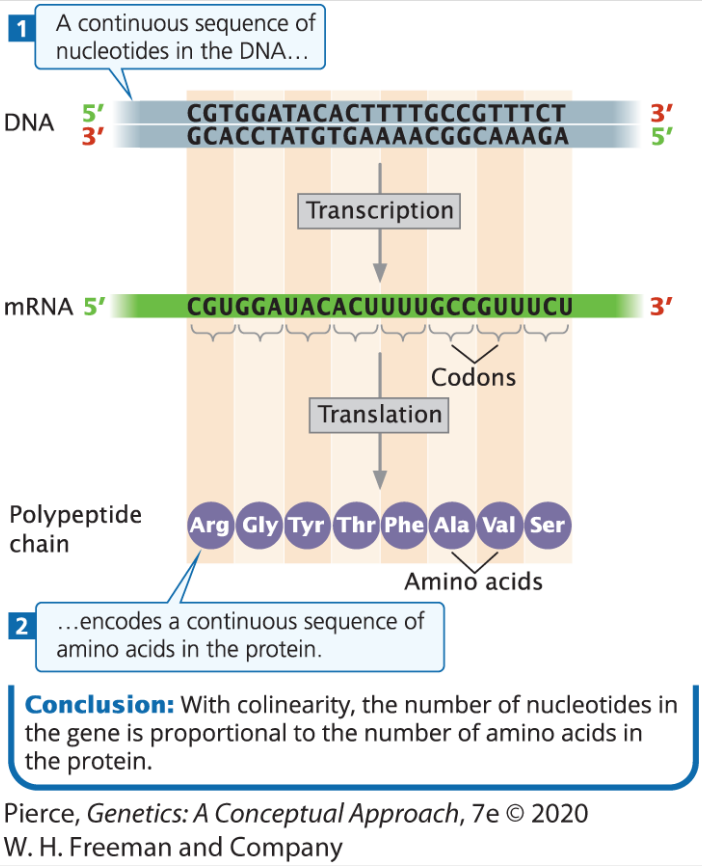
14.1 Many Genes Have Complex Structures

What is a gene? As noted in Chapter 3, the definition of a gene often changes as we explore different aspects of heredity. A gene was defined in Chapter 3 as an inherited factor that determines a characteristic. This definition may have seemed vague because it says only what a gene does, not what a gene is. Nevertheless, this definition was appropriate at the time because our focus was on how genes influence the inheritance of traits. We did not have to consider the physical nature of the gene in learning the rules of inheritance.

Knowing something about the chemical structure of DNA and the process of transcription now enables us to be more precise about what a gene is. Chapter 10 described how genetic information is encoded in the base sequence of DNA: a gene consists of a set of DNA nucleotides. But how many nucleotides constitute a gene, and how is the information in those nucleotides organized? In 1902, Archibald Garrod correctly suggested that genes encode proteins. Proteins are made of amino acids, so a gene contains nucleotides that specify amino acids. Therefore, for many years, the working definition of a gene was a set of nucleotides that specifies the amino acid sequence of a protein. As geneticists learned more about the structure of genes, however, it became clear that this concept of a gene was an oversimplification.

Gene Organization

Early work on gene structure was carried out largely through the examination of mutations in bacteria and viruses. This research led Francis Crick to propose in 1958 that genes and proteins are **colinear**—that there is a direct correspondence between the nucleotide sequence of DNA and the amino acid sequence of a protein (**Figure 14.1**). The concept of colinearity suggests that the number of nucleotides in a gene should be proportional to the number of amino acids in the protein encoded by that gene. In a general sense, this concept is true for genes found in bacterial cells and in many viruses, although these genes are slightly longer than would be expected if colinearity were strictly applied because the mRNAs encoded by the genes contain sequences at their ends that do not specify amino acids. At first, eukaryotic genes and proteins were also assumed to be colinear, but there were hints that eukaryotic gene structure is fundamentally different. Eukaryotic cells were found to contain far more DNA than is required to encode proteins (see Chapter 11). Furthermore, many large RNA molecules observed in the nucleus were absent from the cytoplasm, suggesting that nuclear RNAs undergo some type of change before they are exported to the cytoplasm.



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14.1 The concept of colinearity suggests that a continuous sequence of nucleotides in DNA encodes a continuous sequence of amino acids in a protein.

Bacterial genes are colinear with proteins, but eukaryotic genes often are not.

Description

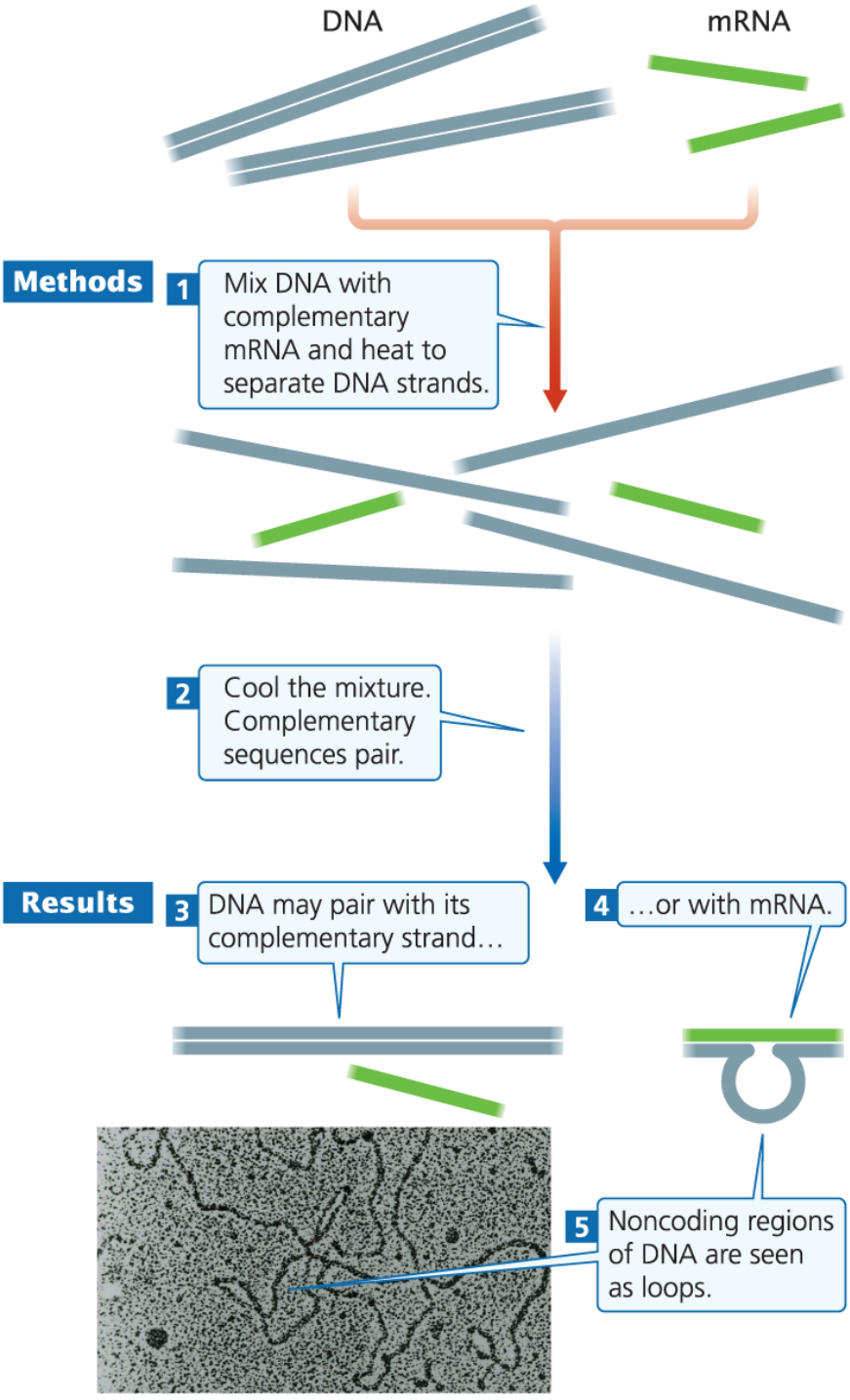
The illustration shows a D N A double strand, at the top. The upper strand runs from 5 prime to 3 prime and the nucleotides are as follows: C, G, T, G, G, A, T, A, C, A, C, T, T, T, T, G, C, C, G, T, T, T, C, T. The lower strand below runs from 3 prime to 5 prime and the nucleotides are as follows: G, C, A, C, C, T, A, T, G, T, G, A, A, A, A, C, G, G, C, A, A, A, G, A. A caption reads, a continuous sequence of nucleotides in the D N A. Transcription occurs and an m R N A transcript running from 5 prime to 3 prime is obtained below. The codons formed after transcription along with the corresponding amino acids formed after translation are as follows: C G U (A r g); G G A (G l y); U A C (T y r); A C U (T h r); U U U (P h e); G C C (A l a); G U U (V a l); U C U (S e r). A caption reads, encodes a continuous sequence of amino acids in the protein. All these amino acids formed are arranged to form

a large polypeptide chain. A caption below reads, With colinearity, the number of nucleotides in the gene is proportional to the number of amino acids in the protein.

Most geneticists were nevertheless surprised by the announcement in the 1970s that not all genes are continuous. Researchers observed four coding sequences in a gene from a eukaryote-infecting virus that were interrupted by nucleotides that did not specify amino acids. This discovery was made when the viral DNA was hybridized with the mRNA transcribed from it and the hybridized structure was examined using an electron microscope (**Figure 14.2**). The DNA was clearly much longer than the mRNA because regions of DNA looped out from the hybridized molecules. These regions of DNA contained nucleotide sequences that were absent from the coding nucleotides in the mRNA. Many other examples of interrupted genes were subsequently discovered; it quickly became apparent that most eukaryotic genes consist of stretches of coding and noncoding nucleotides.

Experiment

Question: Is the coding sequence in a gene always continuous?



Conclusion: Coding sequences in a gene may be interrupted by noncoding sequences.

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From Susan M. Berget, Claire Moore, and Phillip A. Sharp,
Proc. Nati. Acad. Sci. USA Vol. 74, No. 8, pp. 3171–3175,
Fig. 4g August 1977 Biochemistry.

14.2 The noncolinearity of eukaryotic genes was discovered by hybridizing DNA with the mRNA transcribed from it.

Description

The illustration begins with a question that reads, Is the coding sequence in a gene always continuous? The illustration shows 2 double-stranded, larger, D N A molecules and 2 single-stranded, relatively small, m R N A molecules. The illustration shows methods below where D N A is mixed with complementary R N A and heated. A caption reads, mix D N A with complementary m R N A and heat to separate D N A strands. In the next step below, the D N A double strands separate and emerge as separate entities. A caption reads, cool the mixture complementary sequences pair. The results below show, the D N A may pair up with complementary D N A strand or may pair up with a complementary m R N A strand such that the non coding area loops out. A caption reads, D N A may pair with its complementary strand or with m R N A. An electron micrograph shows loops of non coding regions. A caption reads, noncoding regions of D N A are seen as loops. A caption below reads, Conclusion: Coding sequences in a gene may be interrupted by non coding sequences.

CONCEPTS

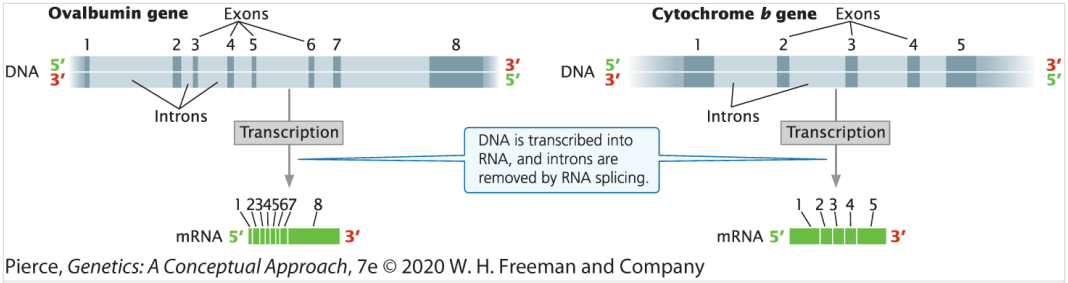
When a continuous sequence of nucleotides in DNA encodes a continuous sequence of amino acids in a protein, the two are said to be colinear. In eukaryotes, not all genes are colinear with the proteins that they encode.

✔ CONCEPT CHECK 1

What evidence indicated that eukaryotic genes are not colinear with the proteins that they encode?

Introns

Many eukaryotic genes contain RNA coding regions called **exons** and noncoding regions called intervening sequences, or **introns**. For example, the gene encoding the protein ovalbumin has eight exons and seven introns; the gene for cytochrome *b* has five exons and four introns (Figure 14.3). The average human gene contains from eight to nine introns. All the introns and exons are initially transcribed into RNA, but during or after transcription, the introns are removed and the exons are joined to yield the mature RNA.



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14.3 The coding sequences of many eukaryotic genes are disrupted by noncoding introns.

Description

The illustration shows a D N A double strand of ovalbumin gene, at the top left, containing 8 exons numbered 1 to 8. Between each of these exons are introns of varying lengths. Transcription occurs and the exons fuse together into one continuous m R N A strand below. The m R N A runs from 5 prime to 3 prime and 1 to 8 exons arranged from left to right. The illustration also shows a D N A double strand for cytochrome small B gene, at the top right, which has 5 exons numbered 1 to 5. Between each of these exons are introns of varying lengths. Transcription occurs and the exons fuse together into one continuous m R N A strand below. The m R N A runs from 5 prime to 3 prime and 1 to 5 exons arranged from left to right. A caption referring to the transcription phase of both genes reads, D N A is transcribed into R N A and introns are removed by R N A splicing.

Introns are common in eukaryotic genes but are rare in bacterial genes. For a number of years after their discovery, introns were thought to be entirely absent from prokaryotic genomes, but they have now been observed in archaea, bacteriophages, and even some bacteria. Introns are present in mitochondrial and chloroplast genes as well as in the nuclear genes of eukaryotes. Among eukaryotic genomes, the sizes and numbers of introns appear to be directly related to organismal complexity: yeast genes contain only a few short introns, *Drosophila* introns are longer and more numerous, and most vertebrate genes are interrupted by long introns. Different classes of eukaryotic genes—those that encode rRNA, tRNA, and proteins—may contain introns. The numbers and sizes of introns vary widely: some eukaryotic genes have no introns, whereas others may have more than 60; intron length varies from fewer than 200 nucleotides to more than 50,000. Introns tend to be longer than exons, and most eukaryotic genes contain more noncoding nucleotides than coding nucleotides.

Geneticists have long debated the evolutionary origin of introns. One idea, called the *intron late hypothesis*, proposes that introns were absent from ancient organisms but were later acquired by eukaryotes. Another idea, termed the *intron early hypothesis*, suggests that early ancestors of bacteria, archaea, and eukaryotes possessed introns that were later lost by prokaryotes and simple eukaryotes. Evidence suggests that introns have been lost *and* gained over

evolutionary time. Many researchers now assume that the earliest eukaryotes possessed introns because divergent eukaryotes have introns in the same positions in their genes, suggesting that these introns were present in the ancestors of all eukaryotes.

There are four major types of introns, differentiated by how the intron is removed from RNA (**Table 14.1**). **Group I introns**, found in some genes of bacteria, bacteriophages, and eukaryotes, are self-splicing: they can catalyze their own removal. **Group II introns** are present in some genes of mitochondria, chloroplasts, archaea, and a few bacteria; they are also self-splicing, but their mechanism of splicing differs from that of the group I introns. **Nuclear pre-mRNA introns** are the best studied; they include introns located in the protein-encoding genes of the eukaryotic nucleus. The splicing mechanism by which these introns are removed is similar to that of the group II introns, but nuclear introns are not self-splicing; their removal requires a spliceosome that includes small nuclear RNAs (snRNAs, discussed in Section 14.2) and a number of proteins. **Transfer RNA introns**, found in tRNA genes of bacteria, archaea, and eukaryotes, use yet another splicing mechanism that relies on enzymes to cut and reseal the RNA. In addition to these major types, there are several other types of introns.

TABLE 14.1 Major types of introns

Type of Intron	Location	Splicing Mechanism

Group I	Genes of bacteria, bacteriophages, and eukaryotes	Self-splicing
Group II	Genes of bacteria, archaea, and eukaryotic organelles	Self-splicing
Nuclear pre-mRNA	Protein-encoding genes in the nucleus of eukaryotes	Spliceosomal
Transfer RNA	tRNA genes of bacteria, archaea, and eukaryotes	Enzymatic

Note: There are also several types of minor introns, including group III introns, twintrons, and archaeal introns.

We'll take a detailed look at the chemistry and mechanics of RNA splicing in Section 14.2. For now, we should keep in mind two general characteristics of the splicing process: (1) the splicing of all pre-mRNA introns takes place in the nucleus; and (2) the order of exons in DNA is usually maintained in the spliced RNA; that is, the coding sequences of a gene may be split up, but they are not usually jumbled up. **› TRY PROBLEM 22**

THINK-PAIR-SHARE Question 1

CONCEPTS

Many eukaryotic genes contain exons and introns. Both are transcribed into RNA, but introns are later removed by RNA processing. The numbers and sizes of introns vary from gene to gene. Introns are common in eukaryotic genes but uncommon in bacterial genes.

✔ CONCEPT CHECK 2

What are the four major types of introns?

The Concept of the Gene Revisited

How does the presence of introns affect our concept of a gene? To define a gene as a sequence of nucleotides that encodes amino acids in a protein no longer seems appropriate because this definition excludes introns, which do not specify amino acids. This definition also excludes the nucleotides that encode the 5′ and 3′ ends of an mRNA molecule, which, as we will see, are required for translation but do not encode amino acids. And defining a gene in these terms also excludes sequences that specify rRNA, tRNA, and other RNAs that do not encode proteins. Given our current understanding of DNA structure and function, we need a more precise definition of a gene.

Many geneticists have broadened the concept of a gene to include all sequences in DNA that are transcribed into a single RNA molecule.

Defined this way, a gene includes all exons, all introns, and those sequences at the beginning and end of the RNA that are not translated into protein. This definition also includes DNA sequences that encode rRNAs, tRNAs, and other types of non-messenger RNA. Some geneticists have expanded the definition of a gene even further to include the entire transcription unit—the promoter, the RNA-coding region, and the terminator. However, new evidence now calls into question even this definition. Research suggests that much of the genome is transcribed into RNA, although it is unclear what, if anything, much of this RNA does. What *is* certain is that the process of transcription is more complex than formerly thought, and defining a gene as a sequence that is transcribed into an RNA molecule is not as straightforward as formerly thought. The more we learn about the nature of genetic information, the more elusive the definition of a gene seems to become.

THINK-PAIR-SHARE Question 2

CONCEPTS

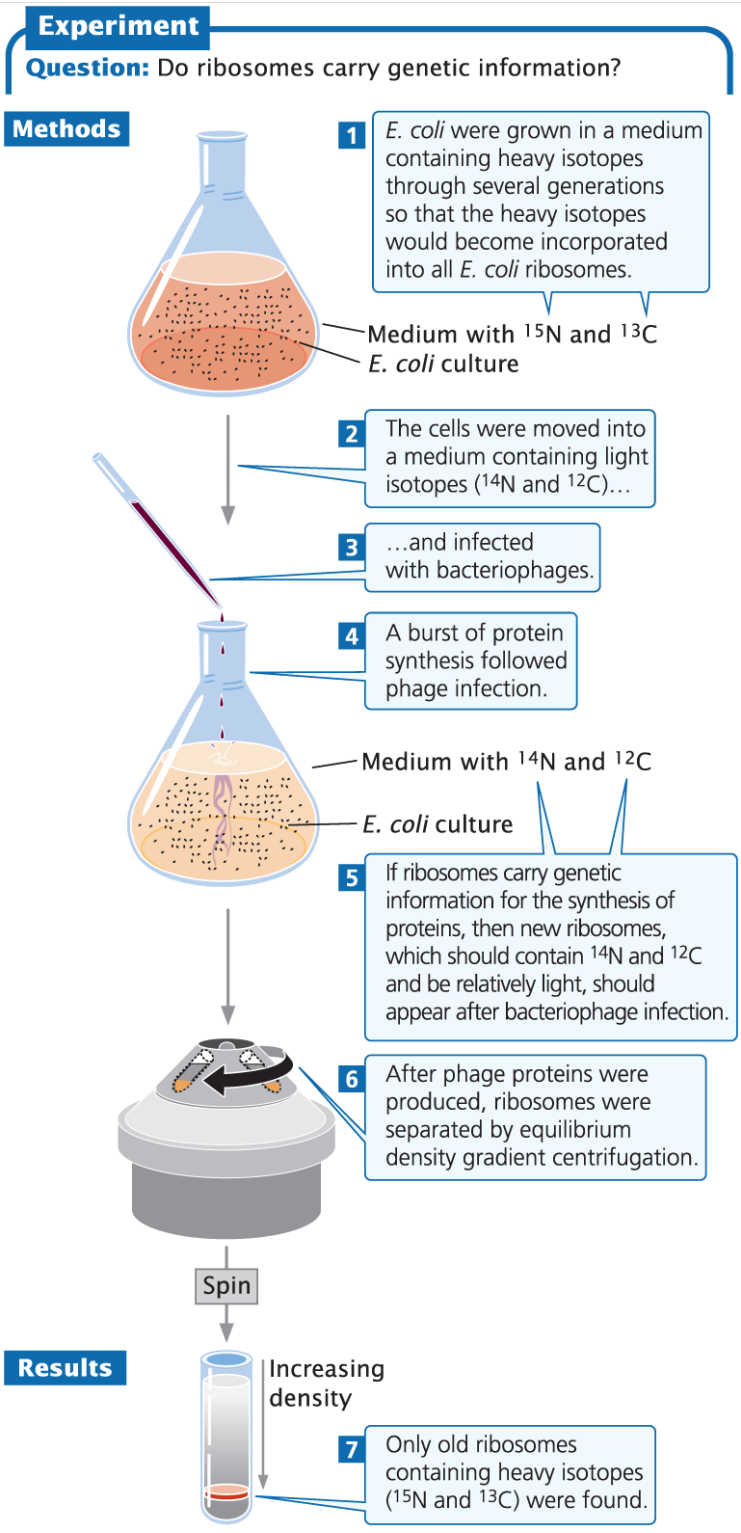
The discovery of introns forced a reevaluation of the definition of a gene. Today, a gene is often defined as a DNA sequence that encodes an RNA molecule or the entire DNA sequence required to transcribe and encode an RNA molecule.

14.2 Messenger RNAs, Which Encode Proteins, Are Modified after Transcription in Eukaryotes

As soon as DNA was identified as the source of genetic information, it became clear that DNA cannot directly encode proteins. In eukaryotic cells, DNA resides in the nucleus, yet protein synthesis takes place in the cytoplasm. Geneticists recognized that an additional molecule must take part in transferring genetic information.

The results of studies of bacteriophage infection conducted in the late 1950s and early 1960s pointed to RNA as a likely candidate for this transport function. Bacteriophages inject their DNA into bacterial cells, where that DNA is replicated (see Chapter 9), and large amounts of phage protein are produced on the bacterial ribosomes. As early as 1953, Alfred Hershey discovered a type of RNA that was synthesized rapidly after bacteriophage infection. Findings from later studies showed that this short-lived RNA had a nucleotide composition similar to that of the phage DNA but quite different from that of the bacterial RNA. These observations were consistent with the idea that RNA was copied from DNA and that this RNA then directed the synthesis of proteins.

At the time, ribosomes were known to be *somehow* involved in protein synthesis, and much of the RNA in a cell was known to be in the form of ribosomes. Were ribosomes the agents by which genetic information was moved to the cytoplasm for the production of protein? Using isotopes of nitrogen and carbon and equilibrium density gradient centrifugation (see Figure 12.2), Sydney Brenner, François Jacob, and Matthew Meselson demonstrated in 1961 that they were not. They showed that new ribosomes are *not* produced during the burst of protein synthesis that accompanies bacteriophage infection (**Figure 14.4**). Thus, ribosomes could not be the carriers of the genetic information needed to produce new phage proteins.



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14.4 Brenner, Jacob, and Meselson demonstrated that ribosomes do not carry genetic information.

Description

The figure begins with a question that reads, Do ribosomes carry genetic information? The illustration shows a flask with a medium containing heavy isotopes (N left superscript 15 and C left superscript 13), at the top. A caption reads, E coli were grown in medium containing heavy isotopes through several generations so that the heavy isotopes would become incorporated into all E coli ribosomes. The cells were then transferred to a flask, placed below, with light isotopes N left superscript 14 and C left superscript 12. The cells are infected with bacteriophages. A caption reads, the cells were moved into medium containing light isotopes N left superscript 14 and C left superscript 12 and infected with bacteriophages. A burst of protein synthesis followed phage infection. If ribosomes carry genetic information for the synthesis of proteins, then new ribosomes, which should contain N left superscript 14 and C left superscript 12 and be relatively light, should appear after bacteriophage infection. The culture is then subjected to centrifugation below. A caption reads, after phage proteins were produced, ribosomes were separated by equilibrium density gradient centrifugation. The culture is spun. The results show the components with increased density settling below in a test tube. A caption reads, only old ribosomes containing heavy isotopes (N left superscript 15 and C left superscript 13) were found. A caption below reads, Conclusion: Ribosomes are not produced in phage reproduction and therefore do not carry genetic information.

In a related experiment, François Gros and his colleagues infected *E. coli* cells with bacteriophages while adding radioactively labeled uracil, which would become incorporated into newly produced phage RNA, to the medium in which they grew. Gros and his colleagues found that the newly produced phage RNA was short-lived, lasting only a few minutes, and was associated with ribosomes but was distinct from them. They concluded that this short-lived RNA carries the genetic information for proteins to the ribosome,

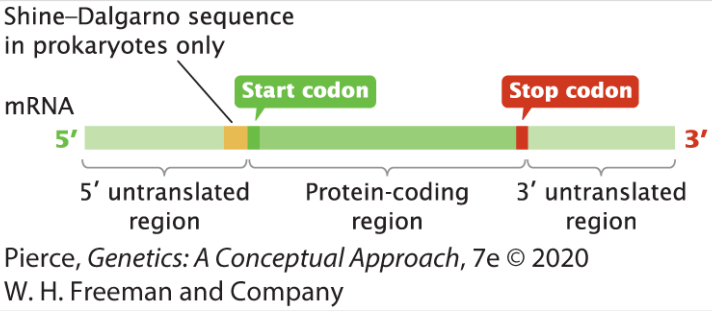
where translation occurs. The term *messenger RNA* was coined for this carrier.

The Structure of Messenger RNA

Messenger RNA functions as the template for protein synthesis. It carries genetic information from DNA to a ribosome, where it helps assemble amino acids in their correct order. In bacteria, mRNA is transcribed directly from DNA, but in eukaryotes, a pre-mRNA (also called the *primary transcript*) is first transcribed from DNA and then processed to yield the mature mRNA. We will reserve the term *mRNA* for RNA molecules that have been processed and are ready to undergo translation.

In an mRNA molecule, each amino acid of a protein is specified by a set of three nucleotides, called a **codon**. Both prokaryotic and eukaryotic mRNAs contain three primary regions (**Figure 14.5**). The first, the **5′ untranslated region (5′ UTR**; sometimes called the *leader*), is a sequence of nucleotides at the 5′ end of the mRNA that does not encode any of the amino acids of a protein. In bacterial mRNA, this region contains a consensus sequence (AGGAGG) called the **Shine–Dalgarno sequence**, which serves as the ribosome-binding site during translation (see Chapter 15); it is found approximately seven nucleotides upstream of the first codon translated into an amino acid (called the start codon). The Shine–Dalgarno sequence is complementary to a sequence found in one of

the RNA molecules that make up the ribosome, and it pairs with that sequence during translation. Eukaryotic mRNA has no equivalent RNA-binding consensus sequence in its 5′ untranslated region. In eukaryotic cells, ribosomes bind to a modified 5′ end of mRNA, as discussed later in this section.



14.5 Three primary regions of mature mRNA are the 5′ untranslated region, the protein-coding region, and the 3′ untranslated region.

Description
The illustration shows an m R N A that runs from 5 prime to 3 prime. The left part represents 5 prime untranslated region. A Shine-Dalgarno sequence is present only in case of prokaryotes. A start codon marks the beginning of protein coding region and a stop codon marks its end. To the right of the stop codon is 3 prime untransalated region.

The second primary region of mRNA is the **protein-coding region**, which comprises the codons that specify the amino acid sequence of the protein. The protein-coding region begins with a start codon and ends with a stop codon.

The third region of mRNA is the **3′ untranslated region** (**3′ UTR**; sometimes called the *trailer*), a sequence of nucleotides at the 3′ end

of the mRNA that is not translated into amino acids. The 3′ UTR affects the stability of mRNA and helps regulate the translation of the mRNA protein-coding sequence. View **Animation 14.1** to see how mutations in different regions of a gene affect the flow of information from genotype to phenotype.

CONCEPTS

Messenger RNA molecules contain three primary regions: a 5′ untranslated region, a protein-coding region, and a 3′ untranslated region. The 5′ and 3′ untranslated regions do not encode any amino acids of a protein but contain information that is important for RNA stability and the regulation of translation.

Pre-mRNA Processing

In bacterial cells, transcription and translation take place simultaneously: while the 3′ end of an mRNA is undergoing transcription, ribosomes attach to the Shine–Dalgarno sequence near the 5′ end and begin translation. Because transcription and translation are coupled, bacterial mRNA has little opportunity to be modified before protein synthesis. In contrast, transcription and translation are separated in both time and space in eukaryotic cells. Transcription takes place in the nucleus, whereas translation takes place in the cytoplasm; this separation provides an opportunity for eukaryotic RNA to be modified before it is translated. Indeed,

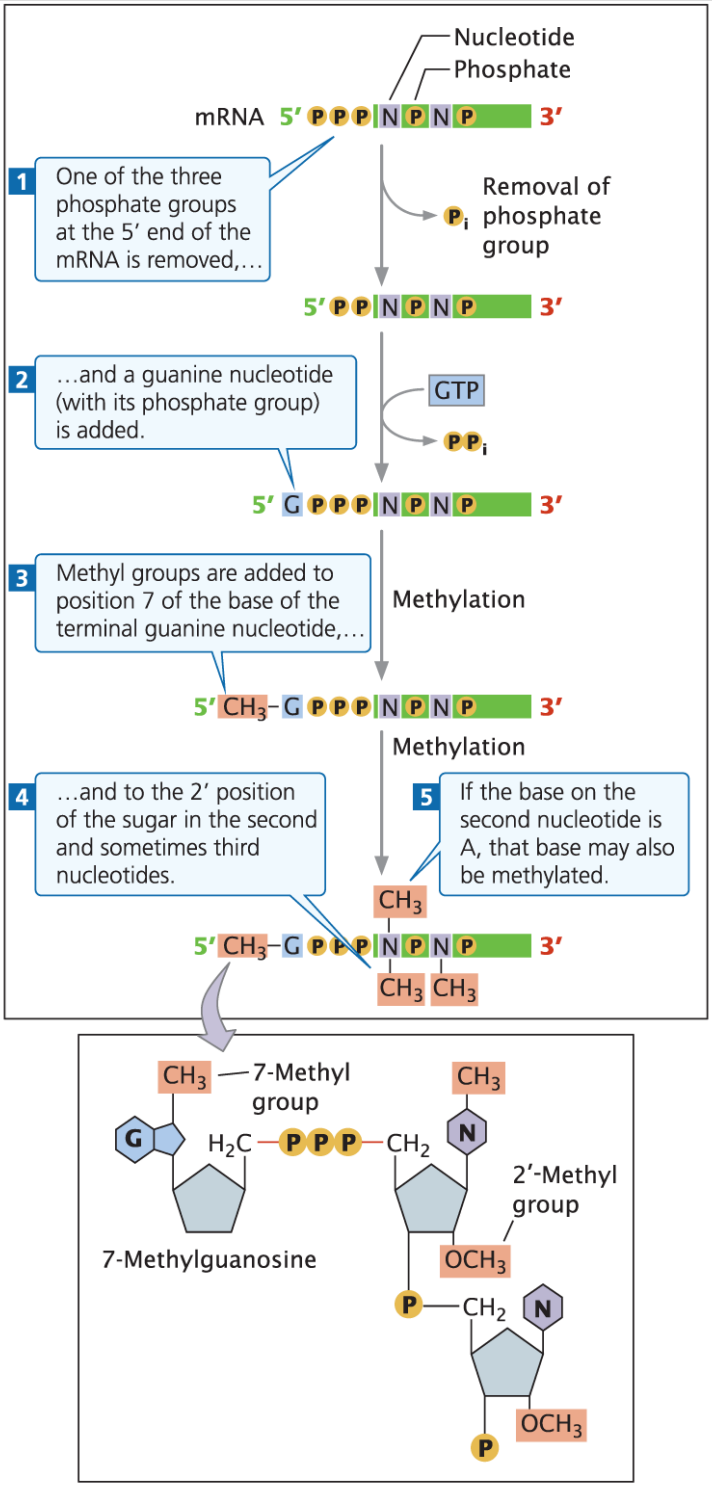
eukaryotic mRNA is extensively altered after transcription. Changes are made to the 5′ end, the 3′ end, and the protein-coding region of the RNA molecule (Table 14.2).

TABLE 14.2 Posttranscriptional modifications to eukaryotic pre-mRNA

Modification	Function
Addition of 5′ cap	Facilitates binding of ribosome to 5′ end of mRNA, increases mRNA stability, enhances RNA splicing
3′ cleavage and addition of poly(A) tail	Increases stability of mRNA, aids in export of mRNA from the nucleus, and facilitates binding of ribosome to mRNA
RNA splicing	Removes noncoding introns from pre-mRNA, facilitates export of mRNA to cytoplasm, allows for multiple proteins to be produced through alternative splicing
RNA editing	Alters nucleotide sequence of mRNA
Internal RNA modifications	Influence splicing, mRNA degradation, and translation

ADDITION OF THE 5′ CAP

One type of modification of eukaryotic pre-mRNA is the addition of a structure called a **5′ cap**. The cap is formed by the addition of an extra guanine nucleotide to the 5′ end of the mRNA and the addition of methyl groups (CH_3) to the base in that guanine, forming 7-methylguanosine. Methyl groups are also added to the 2′-OH group of the sugar of the first and sometimes second nucleotides adjacent to 7-methylguanosine (**Figure 14.6**). If the first adjacent nucleotide has adenine, the adenine base itself may also be methylated. The 7-methylguanosine nucleotide and these additional methyl groups constitute the 5′ cap.



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14.6 Most eukaryotic mRNAs have a 5′ cap. The cap consists of 7-methylguanosine attached to the pre-mRNA by a unique 5′–5′ bond (shown in

detail in the bottom box) and additional methyl groups added to the first few nucleotides.

Description
The illustration shows an m R N A strand running from 5 prime to 3 prime, at the top. The 5 prime end has 3 phosphate molecules and the m R N A has a series of alternate nucleotide and phosphate. Caption 1 reads, one of the three phosphate groups at the 5 prime end of the m R N A is removed. A phosphate group is removed. G T P enters and P P subscript 1 leaves below. The 5 prime end of m R N A has a guanine attached. Caption 2 reads, and a guanine nucleotide (with its phosphate group) is added. Methylation occurs and a methyl group (C H subscript 3) is added to guanine at 5 prime end. Caption 3 reads, methyl groups are added to position 7 of the base of the terminal guanine nucleotide. In the next step below, nucleotides in the m R N A are also methylated. Caption 4 reads, and to the 2 prime position of the sugar in the second and sometimes third nucleotides. If the base on the second nucleotide is A, that base may also be methylated. A magnified image below shows 7-methyl guanosine group bonded to three phosphate groups and other methylated nucleotides in the m R N A.

The addition of the cap takes place rapidly after the initiation of transcription. The cap functions in the initiation of translation, as we'll see in Chapter 15. Cap-binding proteins recognize the cap and attach to it; a ribosome then binds to these proteins and moves downstream along the mRNA until the start codon is reached and translation begins. The presence of a 5′ cap also increases the stability of mRNA and influences the removal of introns.

As noted in the discussion of transcription in Chapter 13, three phosphate groups are present at the 5′ end of all RNA molecules because phosphate groups are not cleaved from the first ribonucleoside triphosphate in the transcription reaction. The 5′ end

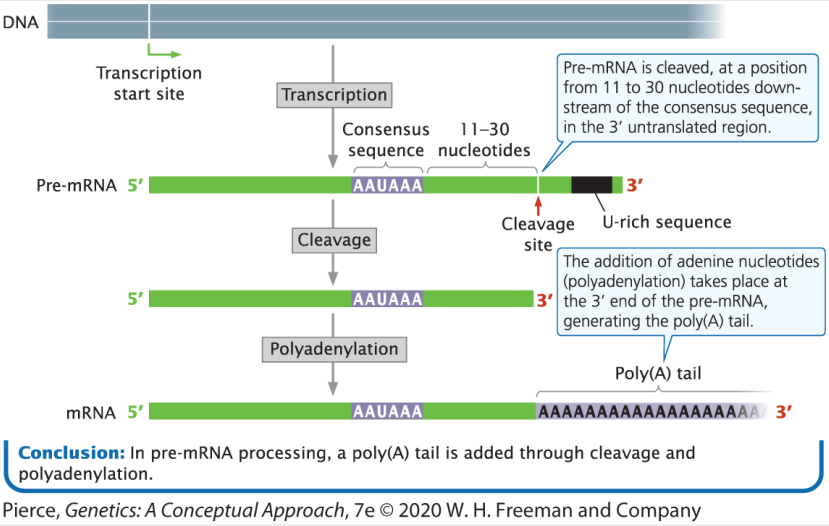
of pre-mRNA can be represented as 5′–PPPNPNP … , in which the letter *N* represents a ribonucleotide and *P* represents a phosphate. Shortly after the initiation of transcription, one of the three 5′ phosphate groups is removed and a guanine nucleotide is added (see Figure 14.6). This guanine nucleotide is attached to the pre-mRNA by a unique 5′ – 5′ bond, which is quite different from the usual 5′ – 3′ phosphodiester bond that joins all the other nucleotides in RNA. One or more methyl groups are then added to the 5′ end of the mRNA; the first of these methyl groups is added to position 7 of the base of the terminal guanine nucleotide, making the base 7-methylguanine. Next, a methyl group may be added to the 2′ position of the sugar in the second and third nucleotides (see Figure 14.6). Rarely, additional methyl groups may be attached to the bases of the second and third nucleotides of the pre-mRNA.

Several different enzymes take part in the addition of the 5′ cap. The initial step is carried out by an enzyme that associates with RNA polymerase II. Because neither RNA polymerase I nor RNA polymerase III have this associated enzyme, RNA molecules transcribed by these polymerases (rRNAs, tRNAs, and some snRNAs) are not capped.

ADDITION OF THE POLY(A) TAIL

A second type of modification to eukaryotic mRNA is the addition of 50 to 250 or more adenine nucleotides at the 3′ end, forming a

poly(A) tail. These nucleotides are not encoded in the DNA but are added after transcription in a process termed *polyadenylation* (Figure 14.7). Many eukaryotic genes transcribed by RNA polymerase II are transcribed well beyond the end of the coding sequence (see Chapter 13); most of the extra material at the 3′ end is then cleaved, and the poly(A) tail is added. More than 1000 nucleotides may be removed from the 3′ ends of some pre-mRNA molecules before polyadenylation.



14.7 Most eukaryotic mRNAs have a 3′ poly(A) tail.

Description

The illustration shows a double stranded D N A molecule at the top with a transcription start site towards its left end. Transcription occurs a pre- m R N A transcript is formed below. From left to right, the regions of the strand are labeled as follows: consensus sequence (A, A, U, A, A, A), 11 to 30 nucleotide region, cleavage site, an unlabeled region, and U-rich sequence. A caption reads, pre-messenger R N A is cleaved at a position from 11 to 30 nucleotides downstream of the consensus sequence, in the 3 prime untranslated region. Cleavage occurs below and the region after the cleavage site is lost. Polyadenylation occurs below and a poly A tail is added at the 3 prime end. The strand is

now labeled as m R N A. A caption reads, the addition of adenine nucleotides or polyadenylation takes place at the 3 prime end of the pre-m R N A, generating the poly A tail. A caption below reads, in pre- m R N A processing, a poly (A) tail is added through cleavage and polyadenylation.

Processing of the 3′ end of pre-mRNA requires sequences (collectively termed the *polyadenylation signal*) located both upstream and downstream of the site where cleavage occurs. The consensus sequence AAUAAA (called the poly(A) consensus sequence) is usually 11 to 30 nucleotides upstream of the cleavage site (see Figure 14.7) and determines the point at which cleavage will take place. A sequence rich in uracil nucleotides (or in guanine and uracil nucleotides) is typically downstream of the cleavage site. A large number of proteins take part in finding the cleavage site and removing the 3′ end. After cleavage has been completed, adenine nucleotides are added without a template to the new 3′ end, creating the poly(A) tail.

The amount of protein produced by a cell depends not only on the amount of mRNA transcribed but also on the stability of the mRNA that is transcribed. Thus, one way in which eukaryotic cells regulate the expression of genes is by controlling degradation of mRNA. The poly(A) tail and the 5′ cap are important in this process, conferring stability on those mRNAs that have these modifications. The 5′ cap and poly(A) tail protect mRNA from degradation by 5′ and 3′ exonucleases, thus increasing the time during which the mRNA remains intact and available for translation. The stability conferred

by the poly(A) tail depends on proteins that attach to the tail and on its length. The poly(A) tail also facilitates attachment of the ribosome to the mRNA and plays a role in export of the mRNA to the cytoplasm.

Poly(U) tails are added to the 3′ ends of some mRNAs, microRNAs, and snRNAs. Poly(U) tails on some mRNAs may facilitate their degradation. › **TRY** PROBLEM 33

INTERNAL MODIFICATIONS

In addition to the 5′ cap and poly(A) tail, eukaryotic mRNAs often contain internal modifications. The most abundant of these is N^6-methyladenosine (m^6A), consisting of the addition of a methyl group to nitrogen to the 6 position of adenine (see Figure 10.11 for the structure of adenine); this modification is often found near stop codons (sequences in mRNA that signal the end of translation) and in 3′ untranslated regions of the mRNA. Researchers have found that the presence of m^6A influences splicing, mRNA degradation, and translation. Other modifications found in mRNA include m^1A (addition of a methyl group to the 1 position of adenine) and modified bases such as inosine and pseudouridine (which also occur in tRNAs).

CONCEPTS

Eukaryotic pre-mRNAs are processed at their 5′ and 3′ ends. A cap, consisting of a modified guanine nucleotide and several methyl groups, is added to the 5′ end. The 5′ cap facilitates the binding of a ribosome, increases the stability of the mRNA, and may affect the removal of introns. Processing at the 3′ end includes cleavage downstream of an AAUAAA consensus sequence and the addition of a poly(A) tail. The poly(A) tail confers stability on the mRNA, plays a role in its export to the cytoplasm, and facilitates attachment of the ribosome. Modifications of internal nucleotides also occur and affect splicing, RNA degradation, and translation.

✔ CONCEPT CHECK 3

Why is it that pre-mRNAs are capped but tRNAs and rRNAs are not?

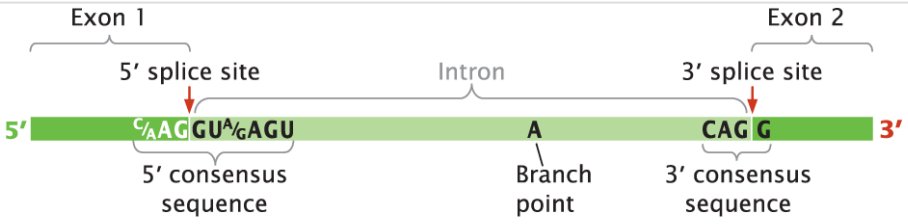
RNA Splicing

The other major type of modification of eukaryotic pre-mRNA is the removal of introns by **RNA splicing**. This modification takes place in the nucleus, before the RNA moves to the cytoplasm.

CONSENSUS SEQUENCES AND THE SPLICEOSOME

Splicing requires the presence of three sequences in the intron. One end of the intron is referred to as the **5′ splice site**, and the other end is the **3′ splice site** (**Figure 14.8**); these splice sites possess short consensus sequences. Most introns in pre-mRNAs begin with GU and end with AG, indicating that these sequences play a crucial role

in splicing. Indeed, changing a single nucleotide at either of these sites prevents splicing.



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14.8 Splicing of pre-mRNA requires consensus sequences. Critical consensus sequences are present at the 5′ splice site and the 3′ splice site. A weak consensus sequence (not shown) exists at the branch point.

Description

The illustration shows a pre-m R N A strand where the intron is located between 2 exons labeled exon 1, on the extreme left, and exon 2, on the extreme right. Two consensus sequences, at the left and right ends are labeled at 5 prime and 3 prime splice sites respectively. The 5 prime consensus sequence, on the left, includes C over A, A, and G before the 5 prime splice site. After the splice site, sequence G, U, A over G, A, G, and U are labeled in the intron region. An adenine group within the intron region is labeled as the branch point. The 3 prime consensus sequence is relatively short and is present on the right side. Nucleotides C, A, G are labeled in the intron region after which 3 prime splice site is marked. A nucleotide G of the 3 prime consensus sequence is labeled in the exon region.

The third sequence important for splicing is the **branch point**, which is an adenine nucleotide that lies 18 to 40 nucleotides upstream of the 3′ splice site (see Figure 14.8). The sequence surrounding the branch point does not have a strong consensus. Deletion or mutation of the adenine nucleotide at the branch point prevents splicing. These sequences—the 5′ splice site, the 3′ splice site, and

the branch point—collectively termed the *splicing code*—determine where splicing will occur. Additional sequences in introns and exons may also affect the process of splicing and which splice sites are used (see Alternative Processing Pathways later in this section).

Splicing takes place within a large structure called the **spliceosome**, which is one of the largest and most complex of all molecular structures. The spliceosome consists of five RNA molecules and almost 300 proteins. The RNA components are small nuclear RNAs (snRNAs) ranging in length from 107 to 210 nucleotides; these snRNAs associate with proteins to form small nuclear ribonucleoprotein particles (snRNPs). Each snRNP contains a single snRNA molecule and multiple proteins. The spliceosome is composed of five snRNPs (U1, U2, U4, U5, and U6) and other proteins not associated with an snRNA.

CONCEPTS

Nuclear pre-mRNA introns contain three consensus sequences critical to splicing: a 5′ splice site, a 3′ splice site, and a branch point. The splicing of pre-mRNA takes place within a large complex called the spliceosome, which consists of snRNAs and proteins.

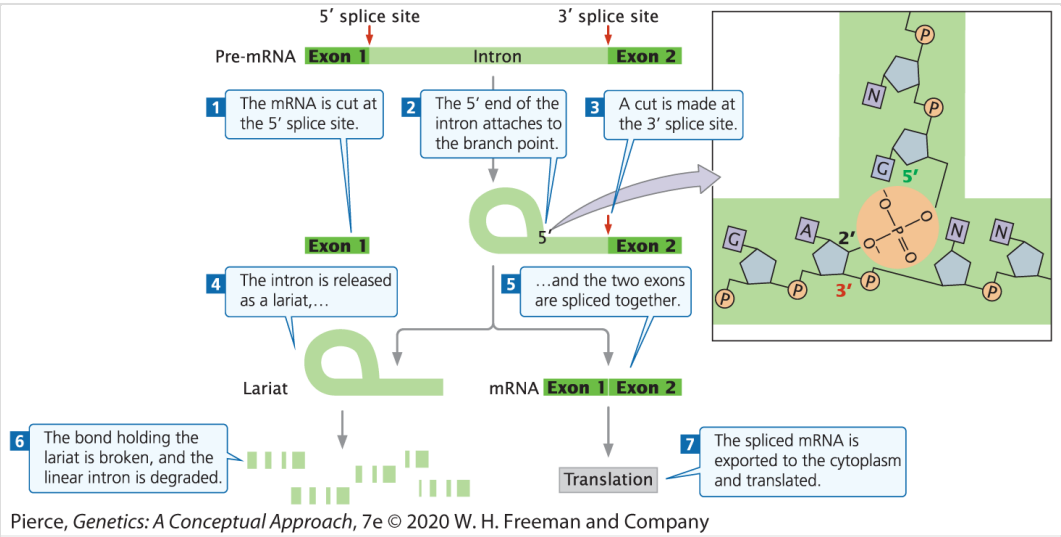
✔ CONCEPT CHECK 4

If a splice site were mutated so that splicing did not take place, what would be the effect on the mRNA?

a. It would be shorter than normal.
b. It would be longer than normal.
c. It would be the same length but would encode a different protein.

THE PROCESS OF SPLICING

Before splicing takes place, an intron lies between an upstream exon (exon 1) and a downstream exon (exon 2), as shown in **Figure 14.9**. Pre-mRNA is spliced in two distinct steps. In the first step of splicing, the pre-mRNA is cut at the 5′ splice site. This cut frees exon 1 from the intron, and the 5′ end of the intron attaches to the branch point, forming a structure called a **lariat**. In this reaction, the guanine nucleotide in the consensus sequence at the 5′ splice site bonds with the adenine nucleotide at the branch point. Both 5′ cleavage and lariat formation in this reaction occur in a single step. As a result, the 5′ phosphate group of the guanine nucleotide becomes attached to the 2′-OH group of the adenine nucleotide at the branch point (see Figure 14.9).



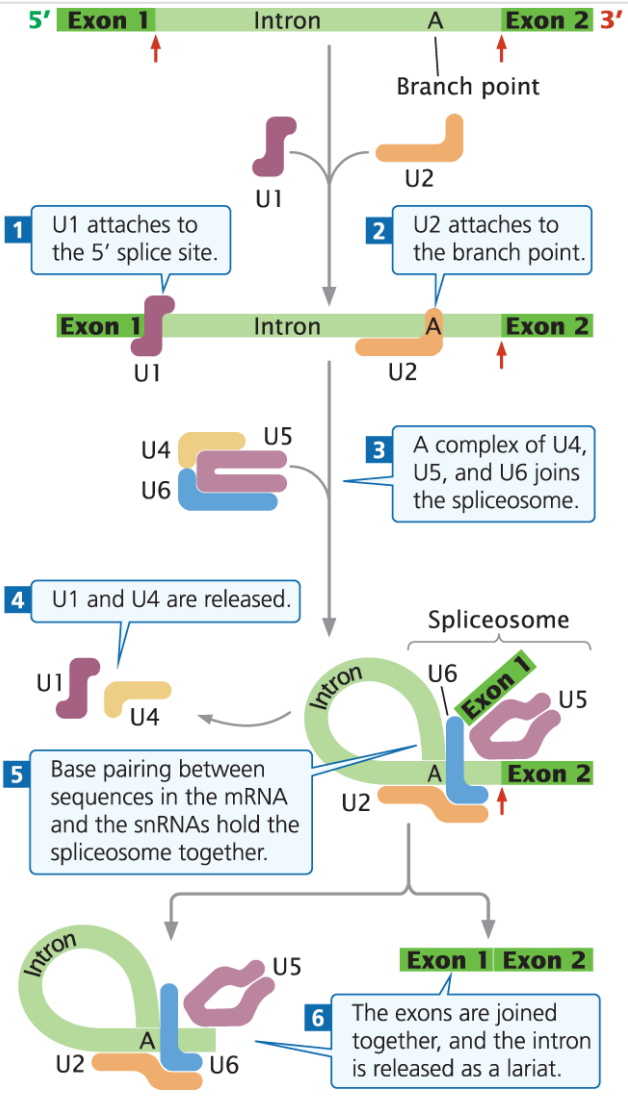
14.9 The splicing of nuclear introns requires a two-step process.

Description
The first picture shows a pre-m R N A at the top. The m R N A has 5 prime splice site after exon 1, on the extreme left, and 3 prime splice site before exon 2, on the extreme right. In between both the splice sites is the intron. In the next step below, m R N A is cut at the 5 prime splice site and the 5 prime end of the intron folds to attach at the branch point. A cut is also made at the 3 prime splice site. A caption reads, the m R N A is cut at the 5 prime splice site. The 5 prime end of the intron attaches to the branch point. A cut is made at the 3 prime splice site. A magnified image on the right shows a phosphate from a nucleotide of the 5 prime intron end bonded to a sugar molecule with adenine base at 2 prime carbon. In the next step below, the intron is folded and the exon 2 yields a lariat and an m R N A with two exons arranged adjacent to one another. A caption reads, the intron is released as a lariat and the two exons are spliced together. In the next step, the lariat is degraded into fragments and the m R N A with exon 1 and exon 2 undergo translation below. A caption reads, the bond holding the lariat is broken, and the linear intron is degraded. The spliced m R N A is exported to the cytoplasm and translated.

In the second step of RNA splicing, a cut is made at the $3'$ splice site, and simultaneously, the $3'$ end of exon 1 becomes covalently attached (spliced) to the $5'$ end of exon 2. The intron is released as a lariat. Eventually, a lariat debranching enzyme breaks the bond at the branch point, producing a linear intron that is rapidly degraded by nuclear enzymes. The mature mRNA, consisting of the exons spliced together, is exported to the cytoplasm, where it is translated.

These splicing reactions take place within the spliceosome, which assembles on the pre-mRNA in a step-by-step fashion (Figure 14.10). A crucial feature of the assembly process is a series of interactions between the snRNAs of the spliceosome and between the snRNAs and the mRNA. These interactions depend on complementary base pairing between the different RNA molecules and bring the essential

components of the pre-mRNA and the spliceosome close together. Key catalytic steps in the splicing process are carried out by the snRNAs of the spliceosome.



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14.10 RNA splicing takes place within the spliceosome. The spliceosome assembles sequentially.

Description

The illustration shows an R N A strand at the top. The R N A strand has a 5 prime splice site after exon 1, on the extreme left, and 3 prime splice site before exon 2, on the extreme right. In between both the slice sites is an intron which has an A nucleotide marked as the branch point. In the next step below, s n R N P U 1 and U 2 enter the process. U 1 attaches to the 5 prime splice site and U 2 attaches to the branch point. A caption reads, U 1 attaches to the 5 prime splice site. U 2 attaches to the branch point. A complex of U 4, U 5, and U 6 enter the process below. A caption reads, A complex of U 4, U 5, and U 6 joins the spliceosome. In the next step below, U 1 and U 4 are released and the 5 prime end of the intron folds and joins at the branch point. U 2 is attached just under the branch point, U 6 is attached at the end of the intron fold. U 5 separates both the exons in an area labeled spliceosome. A caption reads, U 1 and U 4 are released. Base pairing between sequences in the m R N A and the s n R N A s hold the spliceosome together. In the next step below, both the exons join and the intron along with U 5, U 6, and U 2 is released as a lariat. A caption reads, the exons are joined together and the intron is released as a lariat.

First, snRNP U1 attaches to the 5′ splice site, and then U2 attaches to the branch point. A complex consisting of U4, U5, and U6 (which form a single snRNP) joins the spliceosome. This addition causes a conformational change in the spliceosome: the intron loops over, and the 5′ splice site is brought close to the branch point. U1 and U4 dissociate from the spliceosome, and base pairing occurs between U6 and U2 and between U6 and the 5′ splice site. The 5′ splice site, 3′ splice site, and branch point are now in close proximity, held together by the spliceosome. The two transesterification reactions take place, joining the two exons together and releasing the intron as a lariat. The U6 snRNP catalyzes both transesterification reactions.

THINK-PAIR-SHARE Question 3

Most mRNAs are produced from a single pre-mRNA molecule whose exons are spliced together. However, in a few organisms (principally nematodes and trypanosomes), mRNAs may be produced by splicing together sequences from two or more different RNA molecules; this process is called **trans-splicing**. In another variation of splicing, some long introns are removed in multiple steps through a process called **recursive splicing**.

Many human genetic diseases arise from mutations that affect pre-mRNA splicing; indeed, about 15% of single-base substitutions that result in human genetic diseases alter pre-mRNA splicing. Some of these mutations interfere with recognition of the normal 5′ and 3′ splice sites. Others create new splice sites, as was the case with the mutation that caused the royal hemophilia discussed in the introduction to this chapter. › **TRY PROBLEM 28**

RNA splicing, which takes place in the nucleus, must be done before the RNA can move into the cytoplasm. Incompletely spliced RNAs remain in the nucleus until splicing is complete or until they are degraded. Immediately after splicing, a group of proteins called the exon-junction complex (EJC) is deposited approximately 20 nucleotides upstream of each exon–exon junction on the mRNA. The EJC promotes the export of the mRNA from the nucleus to the cytoplasm.

CONCEPTS

Splicing of nuclear pre-mRNA introns is a two-step process: (1) the 5′ end of the intron is cleaved from the upstream exon and attached to the branch point to form a lariat, and (2) the 3′ end of the intron is cleaved from the downstream exon and the ends of the two exons are spliced together. In the process, the intervening intron is removed. These reactions take place within the spliceosome.

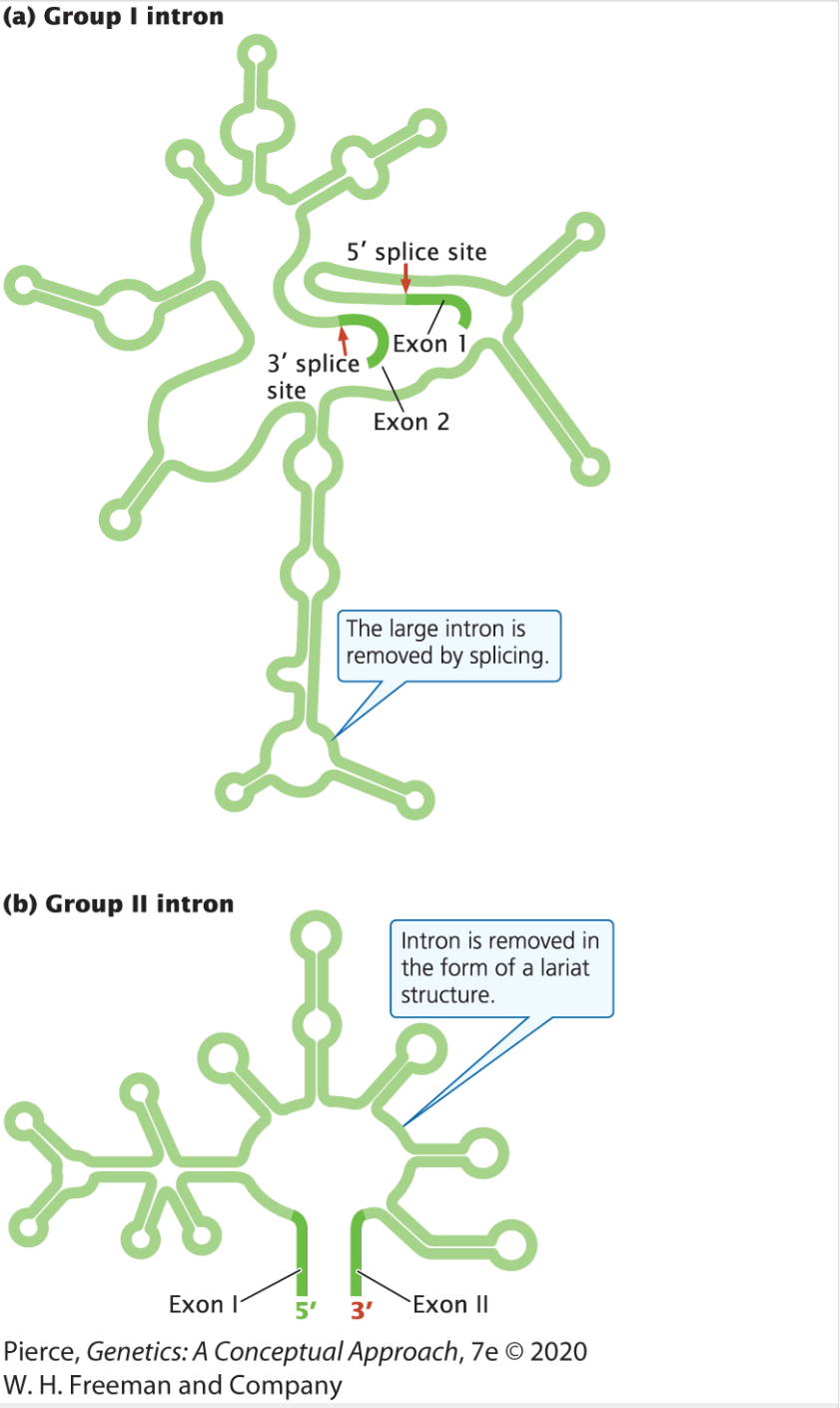
MINOR SPLICING

Some introns in the pre-mRNAs of multicellular eukaryotes are removed by a different process known as minor splicing. Introns that undergo minor splicing have different consensus sequences at the 5′ splice site and branch point and use a minor spliceosome, which consists of a somewhat different set of snRNAs. Some 700–800 genes in the human genome contain introns that undergo minor splicing.

SELF-SPLICING INTRONS

Some introns are self-splicing—they possess the ability to remove themselves from an RNA molecule without the aid of enzymes or other proteins. These self-splicing introns fall into two major categories. Group I introns are found in a variety of genes, including some rRNA genes in protists, some mitochondrial genes in fungi, and even some bacterial and bacteriophage genes. Although the lengths of group I introns vary, all of them fold into a common

secondary structure with nine hairpins (stem-loop structures; Figure 14.11a), which are necessary for splicing.



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14.11 Group I and group II introns fold into characteristic secondary structures.

Description
Part a at the top shows a group 1 intron. It has multiple stem loop structures, which loop around on all sides of the relatively smaller exon 1 and exon 2 sequences. 5 prime splice site is labeled after exon 1 and 3 prime splice site is labeled before exon 2. A caption reads, the large intron is removed by splicing. Part b below shows a group 2 intron. The structure several loops arranged in a semi-circular pattern. Exon 1 and exon 2 arrange parallel to each other as the nearly semi-circular loop extends from exon 1, on the left and ends at exon 2, on the right. A caption reads, the intron is removed in the form of a lariat structure.

Group II introns, present in genes of bacteria, archaea, and eukaryotic organelles, also have the ability to self-splice. All group II introns also fold into secondary structures (Figure 14.11b). The splicing of group II introns is accomplished by a mechanism that has some similarities to the spliceosome-mediated splicing of nuclear pre-mRNA introns, and splicing generates a lariat structure. Because of these similarities, group II introns and nuclear pre-mRNA introns are thought to be evolutionarily related: nuclear introns probably evolved from self-splicing group II introns and later adopted the proteins and snRNAs of the spliceosome to carry out the splicing reaction.

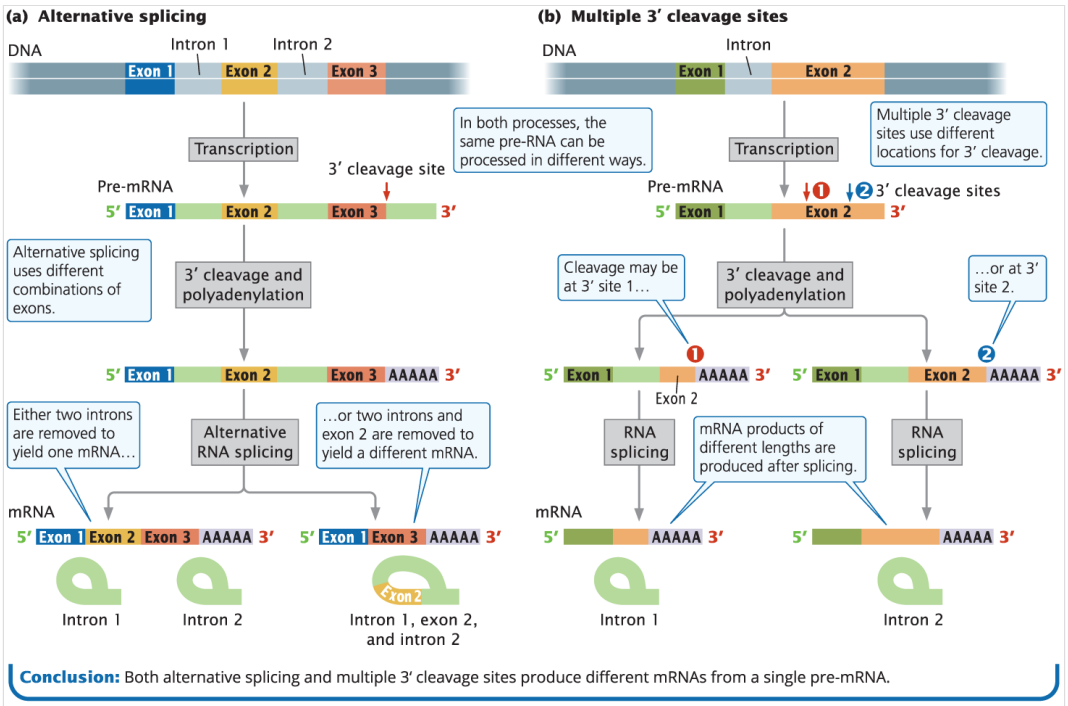
CONCEPTS

Some introns are removed by minor splicing. There are two types of self-splicing introns: group I introns and group II introns. These introns have complex secondary structures that enable them to catalyze their own excision from RNA molecules without the aid of enzymes or other proteins.

Alternative Processing Pathways

A finding that complicates the view of a gene as a sequence of nucleotides that specifies the amino acid sequence of a protein (see The Concept of the Gene Revisited in Section 14.1) is the existence of **alternative processing pathways**. In these pathways, a single pre-mRNA can be processed in different ways to produce alternative types of mRNA, resulting in the production of different proteins from the same DNA sequence.

One type of alternative processing is **alternative splicing**, in which the same pre-mRNA can be spliced in more than one way to yield different mRNAs that are translated into different amino acid sequences and thus different proteins (**Figure 14.12a**). Another type of alternative processing requires **multiple 3′ cleavage sites** (**Figure 14.12b**); in these cases, two or more potential sites for cleavage and polyadenylation are present in the pre-mRNA. In the example in Figure 14.12b, cleavage at the first site produces a relatively short mRNA compared with the mRNA produced through cleavage at the second site. The use of an alternative cleavage site may or may not produce a different protein, depending on whether the site is located before or after the stop codon. Alternative cleavage sites can also change the 3′ untranslated region of the mRNA, which influences the stability and translation of the mRNA.



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14.12 Eukaryotic cells have alternative pathways for processing pre-mRNA. (a) With alternative splicing, pre-mRNA can be spliced in different ways to produce different mRNAs. (b) With multiple 3′ cleavage sites, there are two or more potential sites for cleavage and polyadenylation; use of the different sites produces mRNAs of different lengths.

Description

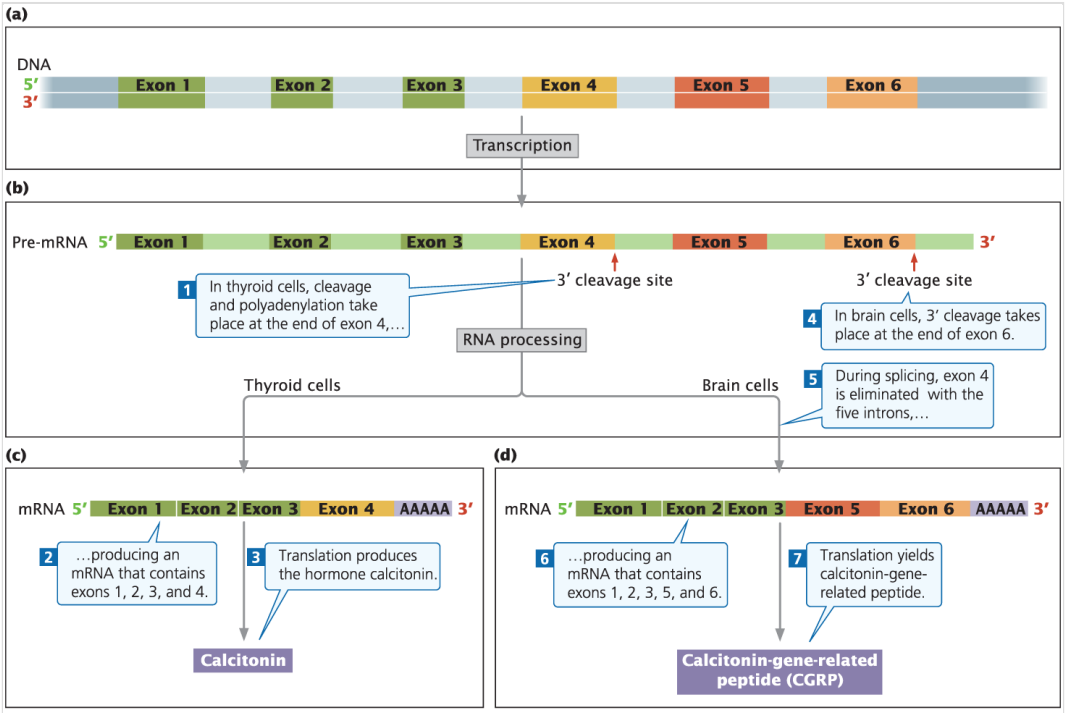
Part a on the left shows alternative splicing. It shows a D N A strand with exons 1 through 3 and introns 1 and 2 between the exons. Transcription occurs and a pre-m R N A strand is formed below with three exons. A 3 prime cleavage site is marked after exon 3. Three prime cleavage and polyadenylation occur. Text reads, alternative splicing uses different combinations of exons. In the next step below, a poly A tail is added to the 3 prime end. Alternate R N A splicing occurs. An m R N A formed below has exon 1, 2 and 3 arranged adjacent to a poly A tail while the intron 1 and intron 2 are removed. A caption reads, either two introns are removed to yield one m R N A. Another possible m R N A formed below has exon 1 and exon 3 with a poly A tail while intron 1, exon 2, and intron 2 are removed. A caption reads, or two introns and exon 2 are removed to yield a different m R N A.

Part b on the right shows a D N A strand with exon 1, intron, and exon 2, from left to right. Text reads, in both processes, the same pre-R N A can be processed in different ways.

Multiple 3 prime cleavage sites use different locations for 3 prime cleavage. Transcription occurs and a pre m R N A is formed below. Two cleavage sites 1 and 2 are labeled within the exon 2 sequence. Three prime cleavage and polyadenylation occur. When the cleavage is at site 1, poly A tail is added after the exon 2. A caption reads, cleavage may be at 3 prime site 1. R N A splicing occurs and an m R N A formed below has exon 1 and a smaller exon 2 attached with a poly A tail. Intron 1 is removed. A caption reads, m R N A products of different lengths are produced after splicing. When cleavage occurs at 3 prime site 2, poly A tail is added after exon 2. A caption reads, or at 3 prime site 2. R N A splicing occurs and an m R N A formed below has exon 1 and a relatively larger exon 2 with a poly A tail. Intron 2 is removed.

Both alternative splicing and multiple 3′ cleavage sites can exist in the same pre-mRNA transcript. An example is seen in the mammalian gene that encodes calcitonin; this gene contains six exons and five introns (**Figure 14.13a**). The entire gene is transcribed into pre-mRNA (**Figure 14.13b**). There are two possible 3′ cleavage sites in the pre-mRNA. In cells of the thyroid gland, 3′ cleavage and polyadenylation take place after exon 4 to produce a mature mRNA consisting of exons 1, 2, 3, and 4 (**Figure 14.13c**). This mRNA is translated into the hormone calcitonin, which is produced by the thyroid gland and regulates calcium concentrations in the blood. In brain cells, the *identical* pre-mRNA is transcribed from DNA, but 3′ cleavage and polyadenylation take place after exon 6, yielding an initial transcript that includes all six exons. During splicing, exon 4 is removed, so only exons 1, 2, 3, 5, and 6 are present in the mature mRNA (**Figure 14.13d**). When translated, this mRNA produces a protein called calcitonin-gene-related peptide (CGRP), which has an amino acid sequence quite different from that of calcitonin. CGRP causes dilation of blood vessels and can function in the transmission

of pain. Some research suggests that CGRP is involved in the development of migraine headaches. Alternative splicing may produce different combinations of exons in the mRNA, but the order of the exons is not usually changed.



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14.13 Pre-mRNA encoded by the gene for calcitonin undergoes alternative processing.

Description

Part a at the top shows a D N A molecule with exons 1 through 6 labeled, from left to right. Transcription occurs and part b below shows pre m R N A molecule with exons 1 through 6. Two 3 prime cleavage sites are marked: one after exon 4 and one after exon 6. R N A processing occurs and an arrow splits, with the left arrow pointing to part c showing the m R N A molecule in thyroid cells. The arrow pointing right shows part d below and the m R N A strand in brain cells. A caption reads, in thyroid cells, cleavage and polyadenylation takes place at the end of exon 4. In brain cells, cleavage at the 3 prime end takes place at the end of exon 6. Part c at bottom left shows m R N A produced in the case of thyroid cells which has exon 1, exon 2, exon 3, and exon 4 arranged adjacent and a poly A tail at 3

prime end. A caption reads, producing an m R N A that contains exons 1, 2, 3, and 4. Translation occurs and hormone calcitonin is produced below. A caption reads, translation produces the hormone calcitonin. Part d shows m R N A produced in the case of brain cells. A caption reads, during splicing, exon 4 is eliminated with the five introns. The m R N A produced has exon 1, exon 2, exon 3, exon 5, exon 6 arranged adjacent with a poly A tail at the 3 prime end. A caption reads, producing an m R N A that contains exons 1, 2, 3, 5, and 6. Translation occurs and calcitonin-gene-related peptide (C G R P) is produced below.

Alternative splicing is controlled by proteins and ribonucleoprotein particles that bind to sites within introns and exons and determine which splice and cleavage sites are used. Alternative processing of pre-mRNAs is common in multicellular eukaryotes. For example, researchers estimate that the products of more than 95% of all human genes with multiple exons undergo alternative splicing and that 50% of human genes have multiple $3'$ cleavage sites. Often the form of splicing differs between human tissues: human brain and liver tissues have more alternatively spliced mRNAs than do other tissues. Sometimes splicing even varies from one person to another. Alternative processing pathways also contribute to gene regulation, as will be discussed in Chapter 17.

Alternative splicing may play a role in organismal complexity. The complete sequencing of the genomes of numerous organisms (see Chapter 20) has led to the conclusion that an organism's number of genes is not correlated with the organism's complexity. For example, fruit flies have only about 14,000 genes, whereas anatomically simpler nematode worms have 20,500 genes. The plant *Arabidopsis*

thaliana has about 25,700 genes, more than humans have. If anatomically simple organisms have as many genes as complex organisms have, how is developmental complexity encoded in the genome? A possible answer is alternative processing, which can produce multiple proteins from a single gene and is an important source of protein diversity in vertebrates.

Research demonstrates that even closely related species often differ in how their pre-mRNAs are spliced. Alternative splicing appears to evolve relatively quickly and may be responsible for physical and behavioral differences between species, such as humans and chimpanzees, that are phenotypically different but genetically similar. Alternative splicing may therefore have played an important role in speciation (see Chapter 26).

CONCEPTS

Alternative splicing enables exons to be spliced together in different combinations to yield mRNAs that encode different proteins. Multiple 3′ cleavage sites allow pre-mRNA to be cleaved and polyadenylated at different sites.

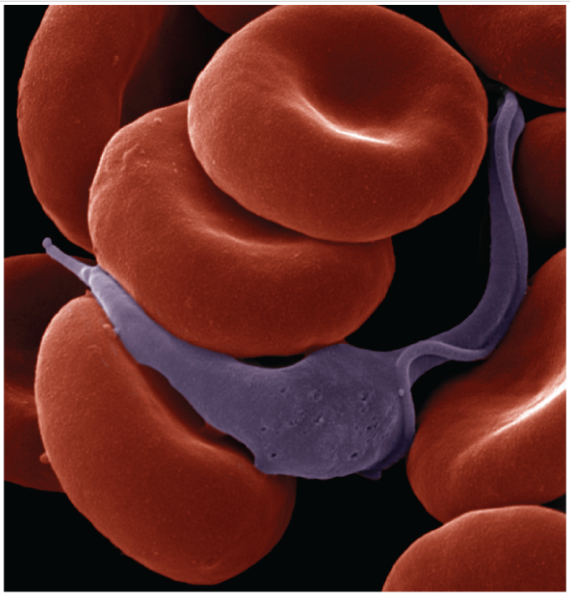
✔ CONCEPT CHECK 5

Multiple 3′ cleavage sites result in

a. multiple genes of different lengths.
b. multiple pre-mRNAs of different lengths.
c. multiple mRNAs of different lengths.
d. All of the above

RNA Editing

The assumption that all information about the amino acid sequence of a protein resides in DNA is violated by a process called RNA editing. In **RNA editing**, the coding sequence of an mRNA molecule is altered after transcription, so that the translated protein has an amino acid sequence that differs from that encoded by the gene. Extensive RNA editing has been found in the mRNA for some mitochondrial genes in trypanosomes (one of which causes African sleeping sickness; **Figure 14.14**). In some mRNAs of these parasites, more than 60% of the sequence is determined by RNA editing. Recent research has demonstrated that extensive RNA editing occurs in RNA molecules associated with neural function in cuttlefish, squid, and octopuses (cephalopods).

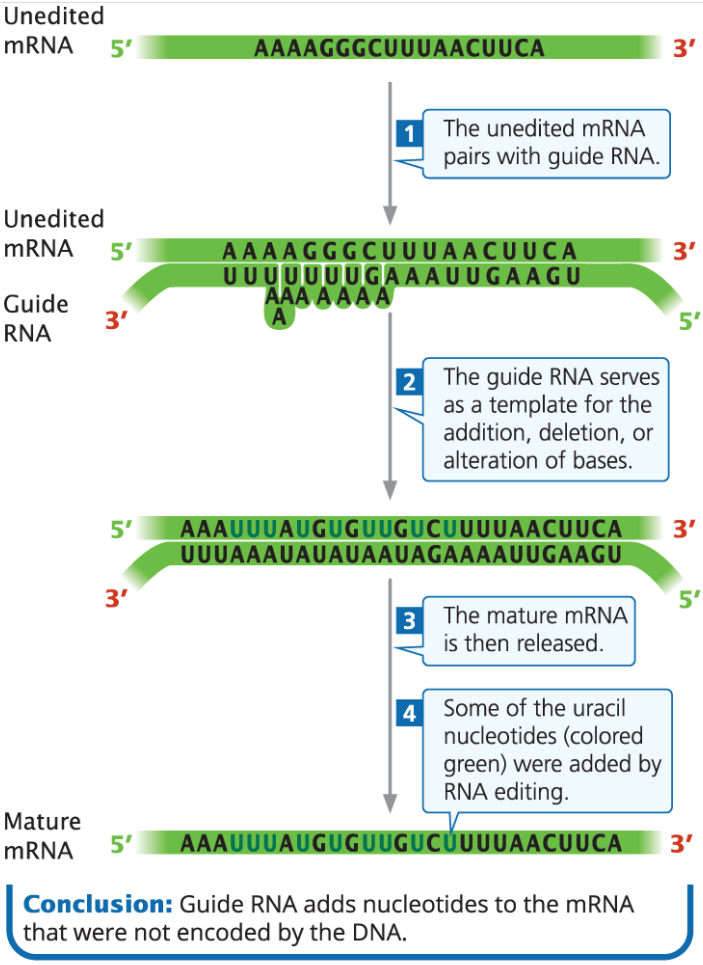


Medical Images RM/Dae Sasitorn.

14.14 *Trypanosoma brucei* causes African sleeping sickness. Messenger RNA produced from mitochondrial genes of this parasite (in purple) undergoes

extensive RNA editing.

If the modified sequence in an edited mRNA molecule doesn't come from a DNA template, then how is it specified? A variety of mechanisms can bring about changes in mRNA sequences. In some cases, molecules called **guide RNAs (gRNAs)** play a crucial role. A gRNA contains sequences that are partly complementary to segments of the unedited mRNA, and the two molecules undergo base pairing at these sequences (**Figure 14.15**). After the mRNA is anchored to the gRNA, the mRNA undergoes cleavage and nucleotides are added, deleted, or altered according to the template provided by the gRNA. In other cases, enzymes bring about base conversion. In humans, for example, a gene is transcribed into an mRNA that encodes a lipid-transporting polypeptide called apolipoprotein-B100, which has 4563 amino acids and is synthesized in liver cells. A truncated form of the protein called apolipoprotein-B48—with only 2153 amino acids—is synthesized in intestinal cells through editing of the apolipoprotein-B100 mRNA. In this editing, an enzyme deaminates a cytosine base, converting it into uracil. This conversion changes a codon that specifies the amino acid glutamine into a stop codon that prematurely terminates translation, resulting in the shortened protein. ❯ **TRY PROBLEM 38**



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14.15 RNA editing is carried out by guide RNAs. The gRNA has sequences that are partly complementary to those of the unedited mRNA, and the two pair. After this pairing, the mRNA undergoes cleavage and new nucleotides are added, with sequences in the gRNA serving as a template. The ends of the mRNA are then rejoined.

Description

The illustration shows an unedited m R N A strand at the top with the following nucleotides: A, A, A, A, G, G, G, C, U, U, U, A, A, C, U, U, C, A. A caption reads, the unedited m R N A pairs with guide R N A. In the next step below, unedited m R N A is bound to the guide m R N A, with the following nucleotides: U, U, U, U, U, U, U, G, A, A, A, U, U, G, A, A, G, U. The nucleotides that do not match (all A's) form bumps in m R N A strand. A caption reads, the guide R N A serves as a template for the addition, deletion, or alteration of bases. In the

next step below, nucleotides have been added to the previously unedited m R N A strand. The m R N A strand now has the following nucleotides: A, A, A, U, U, U, A, U, G, U, G, U, U, G, U, C, U, U, U, U, A, A, C, U, U, C, A. The guide strand has the following nucleotides: U, U, U, A, A, A, U, A, U, A, U, A, A, U, A, G, A, A, A, A, U, U, G, A, A, G, U. A few points show nucleotide G bound to U instead of C. A caption reads, the mature m R N A is then released. The mature m R N A below has the following nucleotides: A, A, A, U (green), U (green), U (green), A, U (green), G, U (green), G, U (green), U (green), G, U (green), C, U (green), U, U, U, A, A, C, U, U, C, A. A caption reads, some of the uracil nucleotides (colored green) were added by R N A editing. A caption reads below, Conclusion: Guide R N A adds nucleotides to the m R N A that were not encoded by the D N A.

CONCEPTS

Individual nucleotides in mRNA may be changed, added, or deleted by RNA editing. The amino acid sequence produced by the edited mRNA is not the same as that encoded by DNA.

✔ CONCEPT CHECK 6

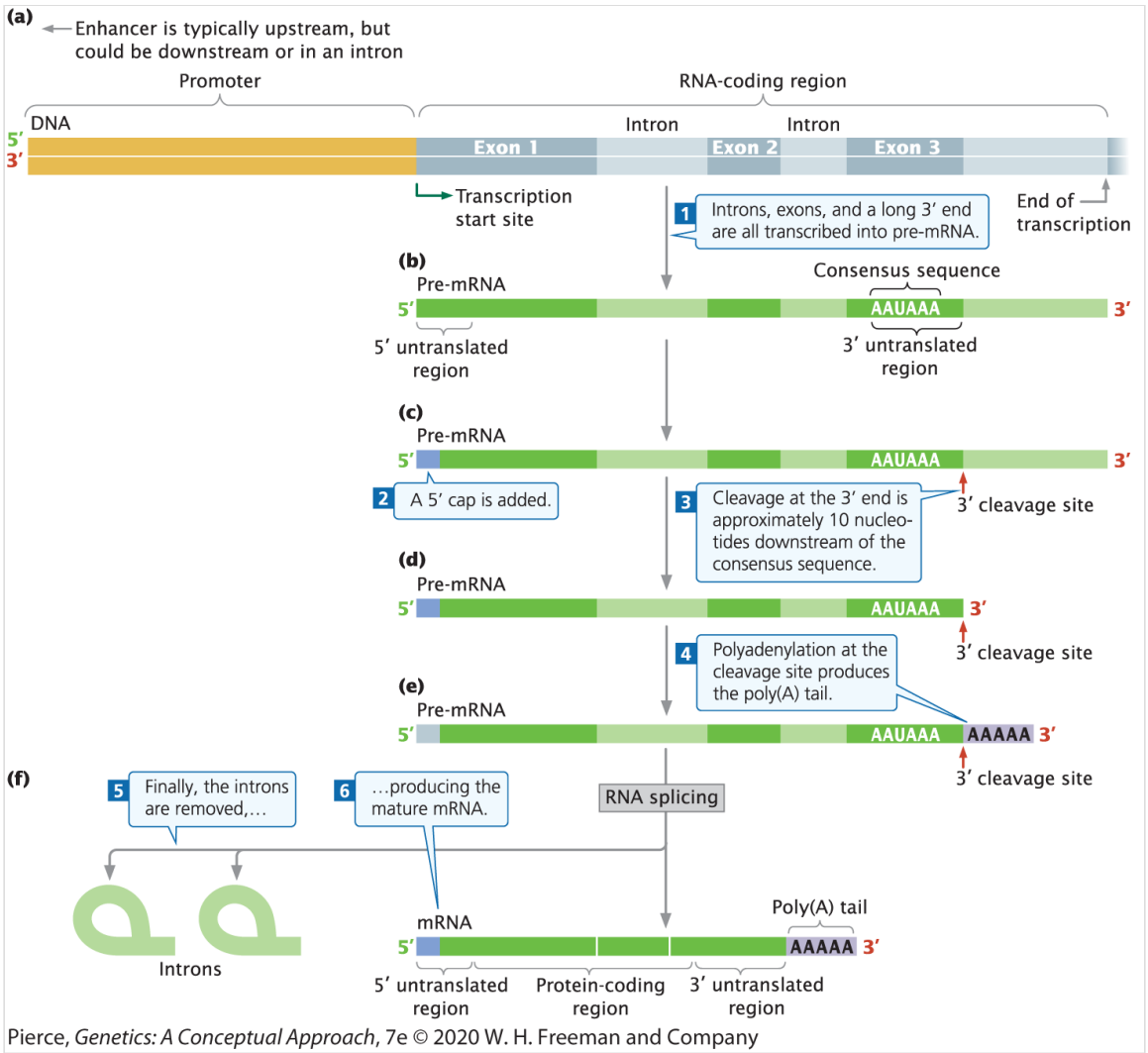
What specifies the modified sequence of nucleotides found in an edited RNA molecule?

CONNECTING CONCEPTS CONNECT

Eukaryotic Gene Structure and Pre-mRNA Processing

Chapters 13 and 14 have introduced a number of different components of genes and RNA molecules, including promoters, 5′ and 3′ untranslated regions, coding sequences (exons), introns, 5′ caps, and poly(A) tails. Let's see how some of these components are combined to create a typical eukaryotic gene and how a mature mRNA is produced from them.

The promoter, which typically lies upstream of the transcription start site, is necessary for transcription to take place but is itself not usually transcribed when protein-encoding genes are transcribed by RNA polymerase II (**Figure 14.16a**). Farther upstream or downstream of the start site, there may be enhancers—DNA sequences involved in regulating transcription.



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14.16 Mature eukaryotic mRNA is produced when pre-mRNA is transcribed and undergoes several types of processing.

Description

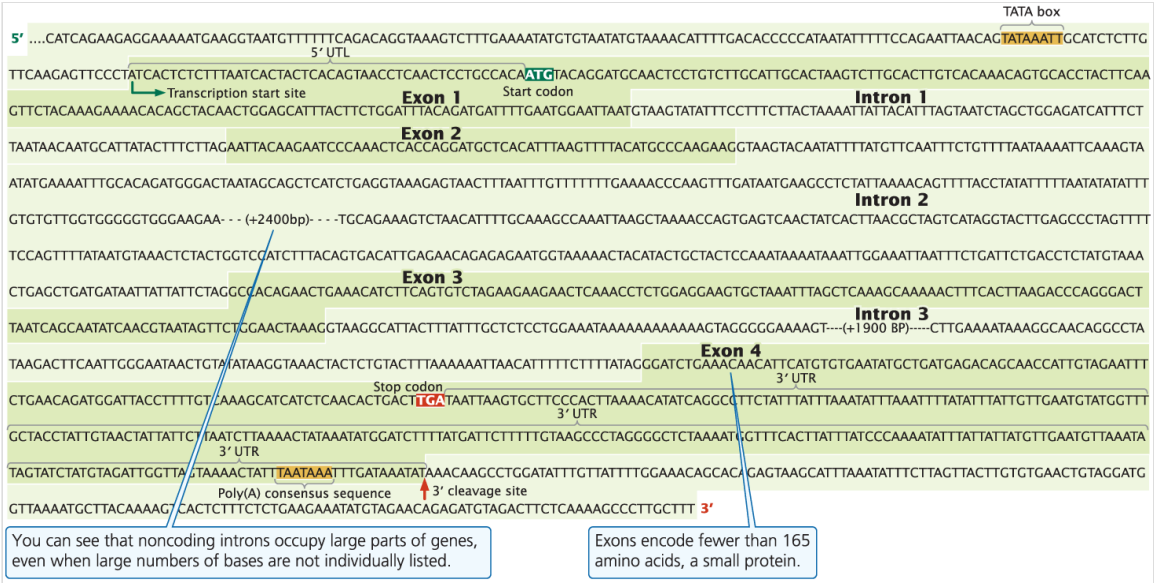
Part a at the top shows a double stranded D N A molecule with the promoter, transcription start site, and R N A-coding region with exons 1 through 3 and introns between them. The extreme right end represents end of transcription. One arrow points toward the left and indicates upstream. Here the enhancer is described as typically being upstream, but could be downstream or in an intron. A caption reads, introns, exons, and a long 3 prime end are all transcribed into pre-m R N A. Part b below shows a pre-m R N A transcript with 5 prime untranslated region and the 3 prime untranslated region containing A, A, U, A, A consensus sequence. Part c below

shows a 5 prime cap added to the 5 prime end of pre- m R N A. A 3 prime cleavage site is marked after exon 3. A caption reads, a 5 prime cap is added. Cleavage at the 3 prime end is approximately 10 nucleotides downstream of the consensus sequence. Part d below shows the pre-m R N A only until the exon 3 as the part on it right is cleaved. Part e below shows a poly A tail added after exon 3 in the pre-m R N A transcript. A caption reads, polyadenylation at the cleavage site produces the poly (A) tail. R N A splicing occurs. R N A splicing occurs. Part f below shows two introns removed such that only exons remain in m R N A formed and a m R N A with 5 prime cap, 5 prime untranslated region, protein-coding region, 3 prime untranslated region and poly A tail at 3 prime end. A caption reads, finally, the introns are removed, producing the mature m R N A.

In transcription, all the nucleotides between the transcription start site and the terminator are transcribed into pre-mRNA, including exons, introns, and a long $3'$ end that is later cleaved from the transcript (**Figure 14.16b**). Notice that the $5'$ end of the first exon contains the $5'$ UTR and that the $3'$ end of the last exon contains the $3'$ UTR.

The pre-mRNA is then processed to yield a mature mRNA. The first step in this processing is the addition of a cap to the $5'$ end of the pre-mRNA (**Figure 14.16c**). Next, the $3'$ end is cleaved at a site downstream of the AAUAAA consensus sequence in the last exon (**Figure 14.16d**). Immediately after cleavage, a poly(A) tail is added to the $3'$ end (**Figure 14.16e**). Finally, the introns are removed to yield the mature mRNA (**Figure 14.16f**). The mRNA now contains $5'$ and $3'$ untranslated regions, which are not translated into amino acids, and the nucleotides that carry the protein-coding sequences. You can explore the consequences of failed RNA processing by viewing and interacting with **Animation 14.2**.

The nucleotide sequence of a small gene (the human interleukin 2 gene), with these components identified, is presented in **Figure 14.17**.



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14.17 This representation of the nucleotide sequence of the gene for human interleukin 2 includes the TATA box, transcription start site, start and stop codons, introns, exons, poly(A) consensus sequence, and 3′ cleavage site.

Description

The illustration shows 5 prime U T R, T A T A box (T, A, T, A, A, A, T, T), transcription start site, start codon (A, T, G), stop codon (T, G, A), introns (1, 2, and 3), exons (1, 2, 3, and 4), poly (A)

consensus sequence (T, A, A, T, A, A, A), 3 prime U T R and 3 prime cleavage site. Introns 2 and 3 have a large number of nucleotides and have an additional 2400 base pairs and 1900 base pairs respectively. A caption reads, you can see the noncoding introns occupy large parts of genes, even when large numbers of bases are not individually listed. Exons encode fewer than 165 amino acids, a small protein.

14.3 Transfer RNAs, Which Attach to Amino Acids, Are Modified after Transcription in Bacterial and Eukaryotic Cells

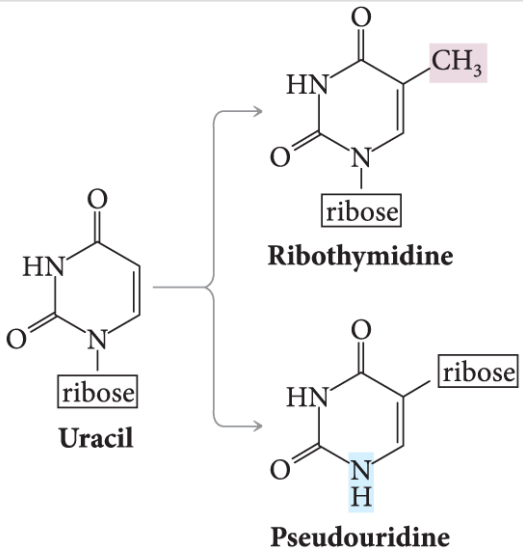
In 1956, Francis Crick proposed the idea of a molecule that transports amino acids to the ribosome and interacts with codons in mRNA, placing the amino acids in their proper order in protein synthesis. By 1963, the existence of such an adapter molecule, called transfer RNA, had been confirmed. Transfer RNA (tRNA) serves as a link between the genetic code in mRNA and the amino acids that make up a protein. Each tRNA attaches to a particular amino acid and carries it to the ribosome, where the tRNA adds its amino acid to the growing polypeptide chain at the position specified by the genetic instructions in the mRNA. We'll take a closer look at the mechanism of this process in Chapter 15.

Each tRNA is capable of attaching to only one type of amino acid. The complex of tRNA plus its amino acid can be written in abbreviated form by adding a three-letter superscript representing the amino acid to the term "tRNA." For example, a tRNA that attaches to the amino acid alanine is written as $tRNA^{Ala}$. Because 20 different amino acids are found in proteins, there must be a minimum of 20 different types of tRNA. In fact, most organisms

possess at least 30 to 40 different types of tRNA, each encoded by a different gene (or, in some cases, multiple copies of a gene) in DNA.

The Structure of Transfer RNA

A unique feature of tRNAs is the occurrence of rare **modified bases**. All classes of RNAs have nucleotides containing the four standard bases (adenine, cytosine, guanine, and uracil) specified by DNA, but tRNAs have nucleotides containing additional bases, including ribothymidine, pseudouridine (which is also occasionally present in snRNAs and rRNA), and dozens of others. The structures of two of these modified bases are shown in **Figure 14.18**.



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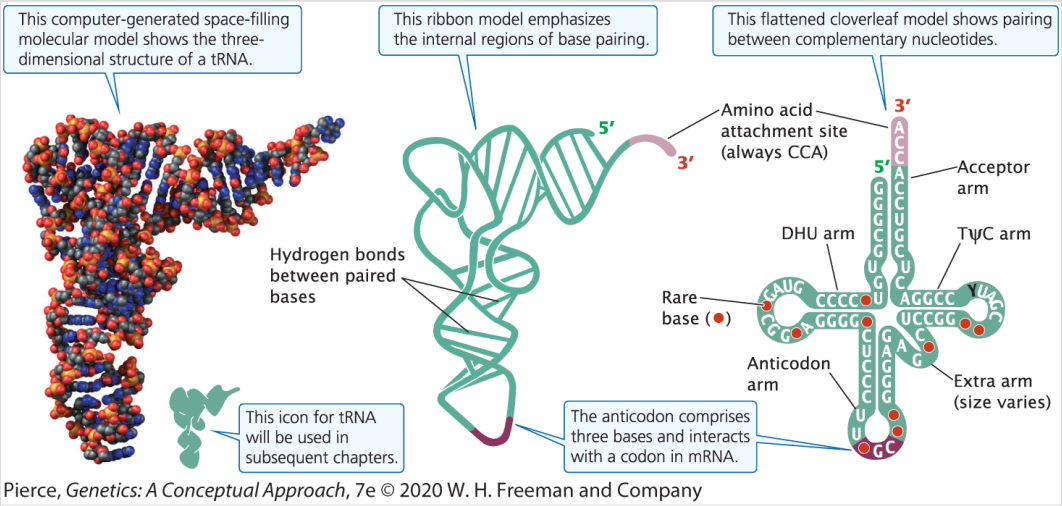
14.18 Ribothymidine and pseudouridine are modified bases found in tRNAs.
These two bases are formed by chemical alteration of uracil.

Description
The illustration shows the conversion of uracil to ribothymidine and pseudouridine. Uracil shows a six-membered ring, in which N 1 is bonded to a ribose. C 2 is a carbonyl group. N 3 is bonded to a hydrogen atom. C 4 is a carbonyl group. C 5 is double bonded to C 6. An arrow from Uracil splits into two: points to Ribothymidine above and Pseudouridine below. Ribothymidine at the top right has a similar structure to that of uracil, except that C 5 is further bonded to a methyl group. Pseudouridine at the bottom right has a similar structure to that of uracil, except that N 1 is now bonded to a hydrogen atom and C 5 is bonded to a ribose.

If there are only four bases in DNA, and all RNA molecules are transcribed from DNA, how do tRNAs acquire these additional bases? Modified bases arise from chemical changes made to the four standard bases after transcription. These changes are carried out by special **tRNA-modifying enzymes**. For example, the addition of a methyl group to uracil creates the modified base ribothymidine. The presence of modified bases affects the structure of the tRNA and how it interacts with the ribosome and with mRNA.

All tRNAs are similar in their secondary structure, a feature that is critical to tRNA function. Most tRNAs are short molecules containing between 74 and 95 nucleotides, some of which are complementary to each other and form intramolecular hydrogen bonds. As a result, each tRNA has a **cloverleaf** structure with four major arms (**Figure 14.19**). If we start at the top and proceed clockwise around the tRNA shown at the right in Figure 14.19, the four major arms are the acceptor arm, the TΨC arm, the anticodon arm, and the DHU arm. Three of the arms (the TΨC, anticodon, and

DHU arms) consist of a hairpin formed by the pairing of complementary nucleotides. A loop lies at the terminus of the hairpin, where there is no nucleotide pairing.



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14.19 All tRNAs possess a common secondary structure, the cloverleaf. The base sequence in the flattened model is for $tRNA^{Ala}$.

Description

The illustration shows space filling molecular model of t R N A on the left. A caption reads, this computer-generated space-filling molecular model shows the 3 D structure of a t R N A. The illustration also shows a ribbon model, at the center, with the internal regions of base-pairing. The hydrogen bonds between the bases are also marked. A caption reads, this ribbon model emphasizes the internal regions of base pairing. Its 3 prime end shows amino acid attachment site (C, C, A). The bottom part comprises an anticodon sequence. A caption reads, the anticodon comprises three bases and interacts with a codon in m R N A. The illustration shows the cloverleaf structure, on the right, where the D H U arm and the T p s i C arm are on the left and right side respectively. A small extra arm of variable size projects out between the T p s i C arm and the anticodon arm at the bottom of the structure, on the right side. Few rare bases are marked all over the structure. At the top of the cloverleaf structure, the amino acid attachment site is labeled at 3 prime end. This region consists of 3 nucleotides, C C A. The bottom part represents anticodon.

Instead of a hairpin, the acceptor arm forms a stem that includes the 5′ and 3′ ends of the tRNA molecule. All tRNAs have the same sequence (CCA) at the 3′ end, where the amino acid attaches to the tRNA, so clearly this sequence is not responsible for specifying which amino acid will attach to the tRNA.

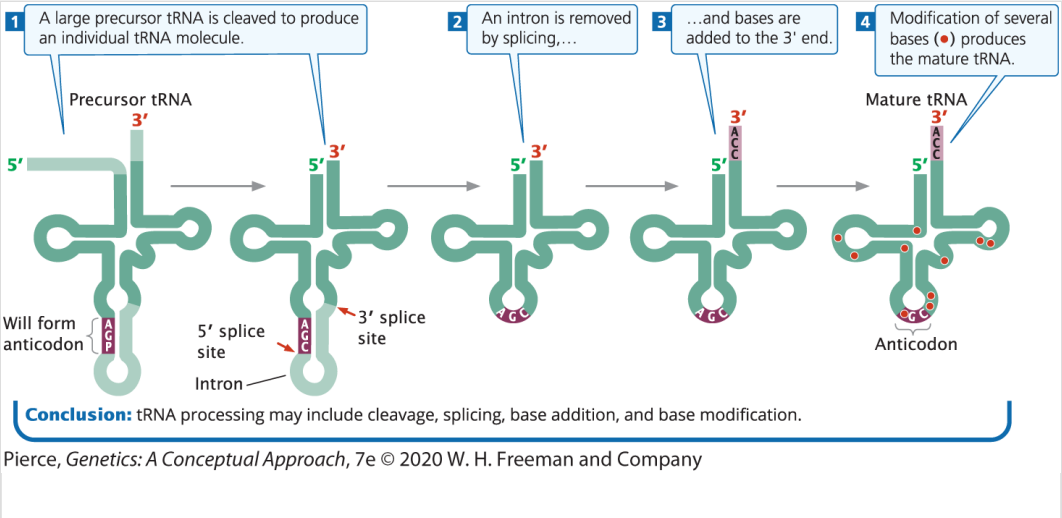
The TΨC arm is named for the bases of three nucleotides in the loop of this arm: thymine (T), pseudouridine (Ψ), and cytosine (C). The anticodon arm lies at the bottom of the tRNA. Three nucleotides at the end of this arm make up the **anticodon**, which pairs with the corresponding codon on mRNA to ensure that the amino acids link in the correct order. The DHU arm is so named because it often contains the modified base dihydrouridine.

Although each tRNA molecule folds into a cloverleaf owing to the complementary pairing of bases, the cloverleaf is not the three-dimensional (tertiary) structure of tRNAs found in the cell. The results of X-ray crystallographic studies have shown that the cloverleaf folds on itself to form an L-shaped structure, as illustrated by the space-filling and ribbon models in Figure 14.19. Notice that the acceptor stem is at one end of the tertiary structure and the anticodon is at the other end.

Transfer RNA Gene Structure and Processing

The genes that produce tRNAs may be in clusters or scattered about the genome. In *E. coli*, the genes for some tRNAs are present in a single copy, whereas the genes for other tRNAs are present in several copies. Eukaryotic cells usually have many copies of each tRNA gene. All tRNA molecules in both bacterial and eukaryotic cells undergo processing after transcription.

In *E. coli*, several tRNAs are usually transcribed together as one large precursor tRNA, which is then cut up into pieces, each containing a single tRNA. Additional nucleotides may then be removed one at a time from the 5′ and 3′ ends of the tRNA in a process known as trimming. Base-modifying enzymes may then change some of the standard bases into modified bases (**Figure 14.20**). In some prokaryotes, the CCA sequences found at the 3′ ends of tRNAs are encoded in the tRNA gene and are transcribed into the tRNA; in other prokaryotes and in eukaryotes, these sequences are added by a special enzyme that adds the nucleotides without the use of any template.



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14.20 Transfer RNAs are processed in both bacterial and eukaryotic cells.
Different tRNAs are modified in different ways. One example is shown here.

Description
The illustration shows cloverleaf structure of precursor t R N A, on the extreme left, which has an intron next to the anticodon (A, G, P). A caption reads, a large precursor t R N A is cleaved to produce an individual t R N A molecule. The next step to its right shows a 5 prime splice site and a 3 prime splice site marked at the beginning and the end of the intron sequence respectively. The next step to its right shows intron is removed by splicing. A caption reads, an intron is removed by splicing. The next step to its right shows bases (A, C, C) are added to the 3 prime end. A caption reads, and bases are added to the 3 prime end. The next step to its right, shows mature R N A is obtained wherein modification of several bases take place. The modified bases are represented as red dots. A caption reads, modification of several bases (red dot symbol) produces the mature t R N A.

There is no generic processing pathway for all tRNAs: different tRNAs are processed in different ways. Eukaryotic tRNAs are processed in a manner similar to bacterial tRNAs: most are transcribed as part of larger precursors that are then cleaved, trimmed, and modified to produce mature tRNAs.

Some eukaryotic and archaeal tRNA genes possess introns of variable length that must be removed in processing. For example, about 40 of the 400 tRNA genes in yeast contain a single intron that is always found adjacent to the 3′ side of the anticodon. These tRNA introns are shorter than those found in pre-mRNA and do not have the consensus sequences found at the intron–exon junctions of pre-mRNAs. The splicing process for tRNA genes is quite different from

the spliceosome-mediated reactions that remove introns from protein-encoding genes.

CONCEPTS

All tRNAs are similar in size and have a common secondary structure known as the cloverleaf. Transfer RNAs contain modified bases and are extensively processed after transcription in both bacterial and eukaryotic cells.

✔ CONCEPT CHECK 7

How are rare bases incorporated into tRNAs?

a. Encoded by guide RNAs
b. Incorporated by chemical changes to one of the standard bases
c. Encoded by rare bases in DNA
d. Encoded by sequences in introns

14.4 Ribosomal RNA, a Component of the Ribosome, Is Also Processed after Transcription

Within ribosomes, the genetic instructions contained in mRNA are translated into the amino acid sequences of polypeptides. Thus, ribosomes play an integral part in the transfer of genetic information from genotype to phenotype. We will examine the role of ribosomes in the process of translation in Chapter 15. Here, we consider ribosome structure and examine how ribosomes are processed before becoming functional.

The Structure of the Ribosome

The ribosome is one of the most abundant molecular complexes in the cell: a single bacterial cell may contain as many as 20,000 ribosomes, and eukaryotic cells possess even more. Ribosomes typically contain about 80% of the total cellular RNA. They are complex structures, each consisting of more than 50 different proteins and RNA molecules (**Table 14.3**). A functional ribosome consists of two subunits, a **large ribosomal subunit** and a **small ribosomal subunit**, each of which consists of one or more RNA molecules and a number of proteins. The sizes of the ribosomes and their RNA components are given in Svedberg (S) units (a measure of how rapidly an object sediments in a centrifugal field). (It is important to note that S units are not additive: combining a 10S structure and a 20S structure does not necessarily produce a 30S structure because the sedimentation rate is affected by the three-dimensional structure of an object as well as by its mass.) The three-dimensional structure of the bacterial ribosome has been elucidated in great detail through X-ray crystallography. More will be said about the ribosome's structure in Chapter 15.

TABLE 14.3 Composition of ribosomes in bacterial and eukaryotic cells

Cell Type	Ribosome Size	Subunits	rRNA Component	Proteins
Bacterial	70S	Large (50S)	23S (2900 nucleotides), 5S (120 nucleotides)	31
		Small (30S)	16S (1500 nucleotides)	21
Eukaryotic	80S	Large (60S)	28S (4700 nucleotides), 5.8S (160 nucleotides), 5S (120 nucleotides)	49
		Small (40S)	18S (1900 nucleotides)	33

Note: The letter *S* stands for "Svedberg unit."

Ribosomal RNA Gene Structure and Processing

The genes for rRNA, like those for tRNA, may be present in multiple copies, and the numbers of copies vary among species (Table 14.4); all copies of an rRNA gene in a species are identical or nearly identical. In bacteria, copies of rRNA genes are dispersed, but in eukaryotic cells, they are clustered, with the genes arrayed in tandem, one after another.

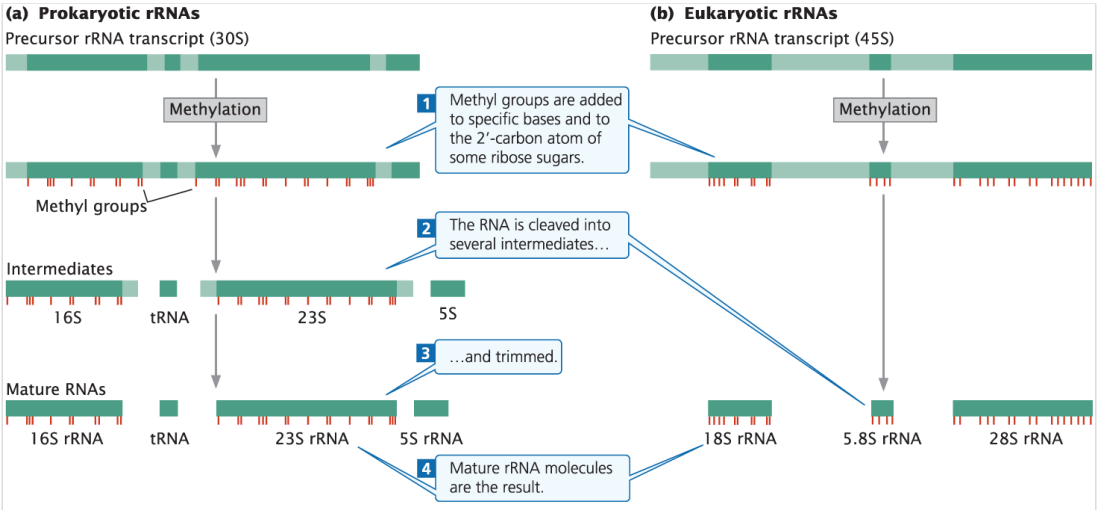
TABLE 14.4 Numbers of rRNA genes in different organisms

Species	Number of Copies of rRNA Genes per Genome

Escherichia coli	7
Yeast	100–200
Human	280
Frog	450

Eukaryotic cells possess two types of rRNA genes: a large gene that encodes 18S rRNA, 28S rRNA, and 5.8S rRNA, and a small gene that encodes 5S rRNA. All three bacterial rRNAs (23S rRNA, 16S rRNA, and 5S rRNA) are encoded by a single type of gene.

Ribosomal RNA is processed after transcription in both bacterial and eukaryotic cells. In *E. coli*, each rRNA gene is transcribed into a 30S rRNA precursor (Figure 14.21a). This 30S precursor is methylated in several places, then cleaved and trimmed to produce 16S rRNA, 23S rRNA, and 5S rRNA, along with one or more tRNAs. A series of enzymes brings about cleavage, methylation, and trimming.



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14.21 Ribosomal RNA is processed after transcription. Prokaryotic rRNA (a) and eukaryotic rRNA (b) are produced from precursor RNA transcripts that are methylated, cleaved, and processed to produce mature rRNAs. Eukaryotic 5S rRNA is transcribed separately from a different gene.

Description
Part a on the left shows the processing of prokaryotic precursor r R N A transcript (30 S). Methylation occurs below and methyl groups are added to some specific bases. A caption reads, methyl groups are added to specific bases and to the 2 prime-carbon atom of some ribose sugars. In the next step below, R N A is cleaved into several intermediates: 16 S, t R N A, 23 S, and 5 S. A caption reads, the R N A is cleaved into several intermediates. In the next step below, the R N A s mature. A caption reads, and trimmed. Mature r R N A molecules are the result. Similarly, part b on the right shows processing of eukaryotic precursor r R N A transcript (45 S). Methylation occurs below and methyl groups are added to some specific bases. A caption reads, methyl groups are added to specific bases and to the 2 prime-carbon atom of some ribose sugars. In the next step below, R N A is cleaved into several intermediates: 18 S r R N A, 5 point 8 S r R N A, and 28 S r R N A. A caption reads, the R N A is cleaved into several intermediates. Mature r R N A molecules are the result.

Eukaryotic rRNAs undergo similar processing (Figure 14.21b). Small nucleolar RNAs (snoRNAs) help cleave and modify eukaryotic rRNAs and assemble them into mature ribosomes. Like the snRNAs taking part in pre-mRNA splicing, snoRNAs associate with proteins to form ribonucleoprotein particles (snoRNPs). The snoRNAs have extensive complementarity to the rRNA sequences in which modification takes place. Interestingly, some snoRNAs are encoded by sequences in the introns of protein-encoding genes. The processing of rRNA and ribosome assembly in eukaryotes, which takes place in the nucleolus, requires over 200 proteins, called assembly factors.

CONCEPTS

A ribosome is a complex structure consisting of several rRNA molecules and many proteins. Each functional ribosome consists of a large and a small subunit. Ribosomal RNAs in both bacterial and eukaryotic cells are modified after transcription. In eukaryotes, rRNA processing is carried out by small nucleolar RNAs.

✔ CONCEPT CHECK 8

What types of changes take place in rRNA processing?

a. Methylation of bases
b. Cleavage of a larger precursor
c. Trimming of nucleotides from the ends of rRNAs
d. All of the above

14.5 Small RNA Molecules Participate in a Variety of Functions

Much evidence suggests that the first genetic material was RNA and that early life was dominated by RNA molecules (see Chapter 13). This early period, when RNA dominated life's essential processes, has been termed the "RNA world." The common perception is that this RNA world died out billions of years ago, when many of RNA's functions were taken over by more stable DNA molecules and more efficient protein catalysts. Within recent years, however, numerous small RNA molecules (most of them 20–30 nucleotides long) that greatly influence many basic biological processes have been discovered. These small RNA molecules play important roles in transcription, translation, chromatin modification, gene expression, development, cancer, and defense against foreign DNA. They are also being harnessed by researchers to study gene function and to treat genetic diseases. The discovery of small RNA molecules has greatly influenced our understanding of how genes are regulated and has demonstrated the importance of DNA sequences that do not encode proteins. These new findings demonstrate that we still live very much in an RNA world.

In this section, we examine RNA interference (which led to the discovery of small RNAs), different types of small RNAs, and the processing of two of these types: microRNAs (miRNAs) and small interfering RNAs (siRNAs). In Chapter 17, we will look further at the

role of small RNAs in controlling gene expression; in Chapter 19, we will see how small RNAs are being used as tools in biotechnology.

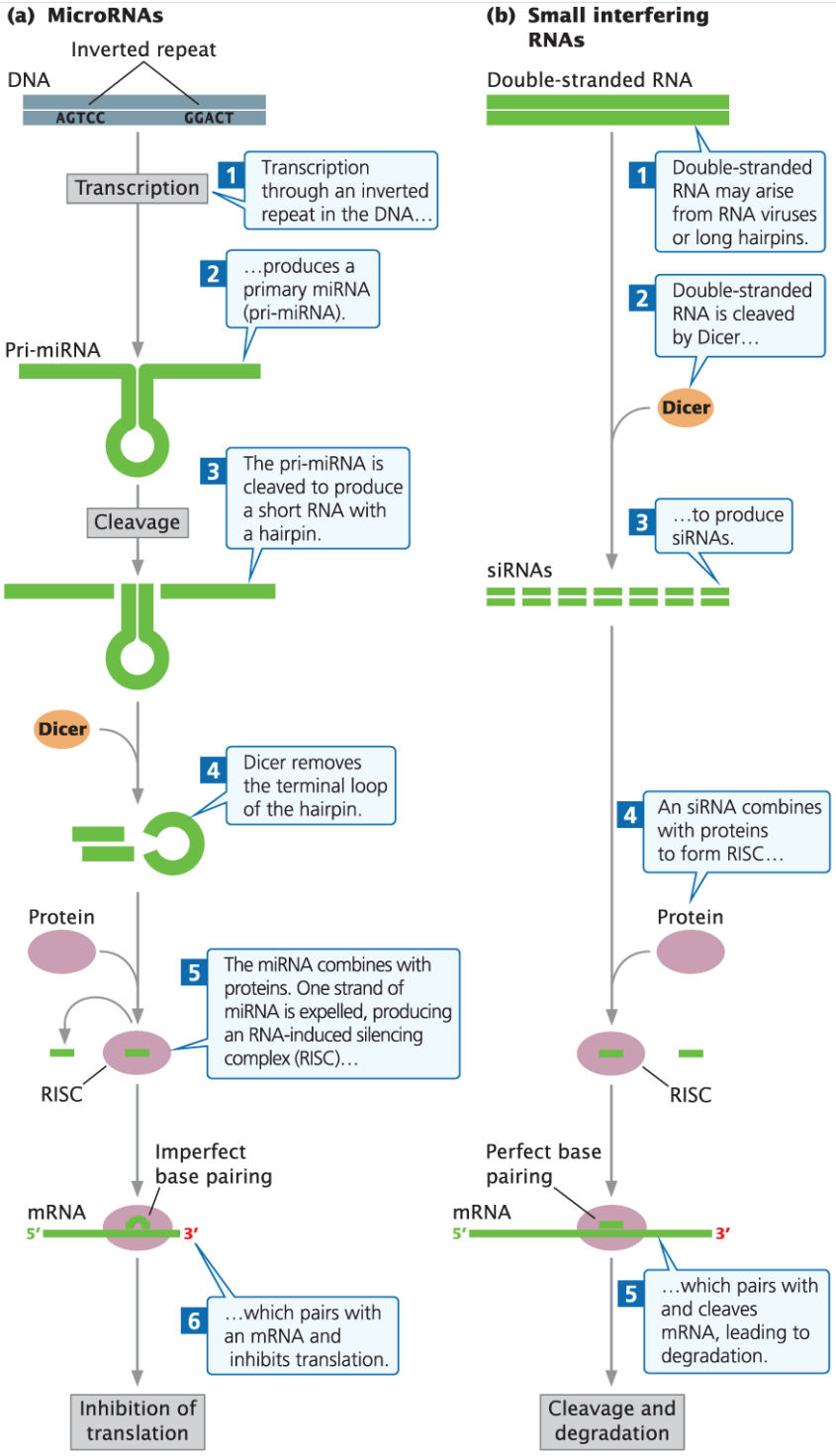
RNA Interference

In 1998, Andrew Fire, Craig Mello, and their colleagues observed a strange phenomenon. They were inhibiting the expression of genes in the nematode *Caenorhabditis elegans* by injecting single-stranded RNA molecules that were complementary to a gene's DNA sequence. Called antisense RNA, such molecules were known to inhibit gene expression by binding to mRNA sequences and inhibiting their translation. However, Fire, Mello, and colleagues found that even more potent gene silencing was triggered when double-stranded RNA was injected into the nematodes. This finding was puzzling, because no mechanism by which double-stranded RNA could inhibit translation was known. Several other, previously described types of gene silencing were also found to be triggered by double-stranded RNA. These initial studies led to the discovery of small RNA molecules that are important in gene silencing. For their discovery of RNA interference, Fire and Mello were awarded the Nobel Prize in physiology or medicine in 2006.

Subsequent research revealed an astonishing array of small RNA molecules with important cellular functions in eukaryotes, which now include at least three major classes: small interfering RNAs (siRNAs), microRNAs (miRNAs), and Piwi-interacting RNAs (piRNAs).

These small RNAs are found in many eukaryotes and are responsible for a variety of different functions, including the regulation of gene expression, defense against viruses, suppression of transposons, and modification of chromatin structure. An analogous group of small RNAs with silencing functions—called CRISPR RNAs (crRNAs)—have been detected in prokaryotes.

RNA interference (RNAi) is a powerful and precise mechanism used by eukaryotic cells to limit the invasion of foreign genes (from viruses and transposons) and to censor the expression of their own genes. RNA interference is triggered by double-stranded RNA molecules, which may arise in several ways (**Figure 14.22**): by the transcription of inverted repeats into an RNA molecule that then base pairs with itself to form double-stranded RNA; by the simultaneous transcription of two different RNA molecules that are complementary to each other and that pair, forming double-stranded RNA; or by infection by viruses that make double-stranded RNA. All of these types of double-stranded RNA molecules are chopped up by an enzyme appropriately called Dicer, resulting in tiny RNA molecules that are unwound to produce siRNAs and miRNAs.



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14.22 MicroRNAs and small interfering RNAs are produced from double-stranded RNAs. Although microRNAs (a) and small interfering RNAs (b) differ in how they originate, they share a number of features, and their functions overlap.

Description
Part a on the left shows a D N A double stranded molecule with inverted repeats (A, G, T, C, C). Transcription occurs and p r i-m i R N A is produced below. The p r i-m i R N A has a hairpin loop in the middle. A caption reads, transcription through an inverted repeat in the D N A produces a primary m i R N A (p r i-m i R N A). Cleavage occurs below and the p r i-m i R N A and the hairpin loop gets separated. A caption reads, the p r i-m i R N A is cleaved to produce a short R N A with a hairpin. A dicer molecule enters the process below and removes the terminal loop of hairpin. A caption reads, dicer removes the terminal loop of the hairpin. A protein molecule enters the process, that leads to expulsion of a strand of m i R N A and formation of an R I S C. A caption reads, the m i R N A combines with proteins. One strand of m i R NA is expelled, producing an R N A-induced silencing complex (R I S C). In the next step below, the complex shows a bump formed due to imperfect pairing with an m R N A. A caption reads, which pairs with an m R N A and inhibits translation. Inhibition of translation occurs below. Part b on the right shows a double stranded R N A molecule. A caption reads, Double-stranded R N A may arise from R N A viruses or long hairpins. A dicer molecule enters the process and 7 s i R N A's are produced below. A caption reads, double-stranded R N A is cleaved by Dicer to produce s i R N A's. A protein molecule enters the process and formation of an R I S C occurs below. A caption reads, an s i R N A combines with proteins to form R I S C. This R I S C complex pairs with an m R N A perfectly. A caption reads, which pairs with and cleaves m R N A, leading to degradation. Cleavage and degradation occurs below.

Some geneticists speculate that RNAi evolved as a defense mechanism against RNA viruses and transposable elements that move through RNA intermediates (see Chapter 18); indeed, some have called RNAi the immune system of the genome. However, RNAi is also responsible for regulating a number of key genetic and developmental processes, including changes in chromatin structure, translation, cell fate and proliferation, and cell death. Geneticists also use the RNAi machinery as an effective tool for blocking the expression of specific genes (see Chapter 19).

Small Interfering RNAs and MicroRNAs

Two abundant classes of RNA molecules that function in RNA interference in eukaryotes are small interfering RNAs and microRNAs. Although these two types of RNA differ in how they originate (**Table 14.5**; see also Figure 14.22), they have a number of features in common, and their functions overlap considerably. Both are about 22 nucleotides long. Small interfering RNAs arise from the cleavage of mRNAs, RNA transposons, and RNA viruses. Some miRNAs are cleaved from RNA molecules transcribed from sequences that encode miRNA only, but others are encoded in the introns and exons of mRNAs. Each miRNA is cleaved from a single-stranded RNA precursor that forms small hairpins, whereas multiple siRNAs are produced from the cleavage of an RNA duplex consisting of two different RNA molecules.

TABLE 14.5 Differences between siRNAs and miRNAs

Feature	miRNA	siRNA
Origin	RNA transcribed from distinct gene	mRNA, transposon, or virus
Cleavage of	Single-stranded RNA that forms short hairpins of double-stranded RNA	RNA duplex or single-stranded RNA that forms long hairpins

Size	21–25 nucleotides	21–25 nucleotides
Action	Degradation of mRNA, inhibition of translation	Degradation of mRNA
Target	Genes other than those from which they were transcribed	Genes from which they were transcribed

Usually, siRNAs have exact complementarity with their target mRNA or DNA sequences, whereas miRNAs often have limited complementarity with their target mRNAs. Small interfering RNAs primarily suppress gene expression by degrading mRNA, while miRNAs often suppress gene expression by inhibiting translation. Finally, miRNAs usually silence genes that are distinct from those from which the miRNAs were transcribed, whereas siRNAs typically silence the genes from which the siRNAs were transcribed. Note, however, that these differences between siRNAs and miRNAs are not hard and fast, and scientists are increasingly finding small RNAs that exhibit characteristics of both. For example, some miRNAs (such as those in plants) have exact complementarity with mRNA sequences and cleave those sequences—characteristics that are usually associated with siRNAs.

Both siRNA and miRNA molecules combine with proteins to form an **RNA-induced silencing complex (RISC)** (see Figure 14.22). Key to the

functioning of RISCs is a protein called Argonaute. The RISC pairs with an mRNA molecule that possesses a sequence complementary to its siRNA or miRNA component, and the RISC then either cleaves the mRNA, leading to its degradation, or represses translation of the mRNA. Some siRNAs also serve as guides for the methylation of complementary sequences in DNA, and others alter chromatin structure, both of which affect transcription. To see how small interfering RNAs and microRNAs affect gene expression, see **Animation 14.3**.

MicroRNAs (but not siRNAs) are encoded within an organism's own genome and are transcribed from miRNA genes that are usually found in regions of noncoding DNA or within the introns of other genes. Thus, miRNAs are often described like other RNA molecules. Most are named miR with a dash and a number, such as miR-33 or miR-140. MicroRNAs have been found in all eukaryotic organisms examined to date, as well as in viruses: they control the expression of genes taking part in many biological processes, including growth, development, and metabolism. Humans have more than 500 distinct miRNAs; scientists estimate that most human mRNAs are regulated by miRNAs.

THINK-PAIR-SHARE Question 4

PROCESSING AND FUNCTION OF miRNAs AND siRNAs

The genes that encode miRNA are transcribed into longer precursors, called primary miRNA (pri-miRNA), that range from several hundred to several thousand nucleotides in length (see Figure 14.22a). The pri-miRNA is then cleaved into one or more smaller RNA molecules, each containing a hairpin. Dicer binds to the hairpin structure and removes the terminal loop. One of the resulting miRNA strands is incorporated into the RISC; the other strand is expelled and degraded. (A) **Animation 14.3** illustrates the process by which miRNAs are produced.

The RISC then attaches to a complementary sequence on the target mRNA, usually in the 3′ UTR of the mRNA. The region of close complementarity between the miRNA and a target mRNA, called the seed region, is quite short, usually only about seven nucleotides long. Because the seed region is so short, each miRNA can potentially pair with sequences on hundreds of different mRNAs. Furthermore, a single mRNA molecule may possess multiple miRNA-binding sites. The inhibition of translation may require binding by several RISC complexes to the same mRNA molecule.

Small interfering RNAs are processed in a similar way (see Figure 14.22b): double-stranded RNA from viruses or long hairpins is cleaved by Dicer to produce siRNAs. An siRNA then combines with proteins to form a RISC. The RISC pairs with sequences on the target mRNA and cleaves it, after which the mRNA is degraded.

THINK-PAIR-SHARE Question 5

CONCEPTS

Small interfering RNAs and microRNAs are tiny RNAs produced when larger, double-stranded RNA molecules are cleaved by the enzyme Dicer. Small interfering RNAs and microRNAs participate in a variety of processes, including mRNA degradation, the inhibition of translation, the methylation of DNA, and chromatin remodeling.

✔ CONCEPT CHECK 9

How do siRNAs and miRNAs target specific mRNAs for degradation or for the repression of translation?

Piwi-Interacting RNAs

Piwi-interacting RNAs (piRNAs), which were discovered in 2001, differ from siRNAs and miRNAs in several ways. They are somewhat longer than siRNAs or miRNAs, consisting of 24 to 31 nucleotides, and are derived from long, single-stranded RNA transcripts. Furthermore, Dicer is not involved in the production of piRNAs.

Piwi-interacting RNAs combine with Piwi proteins, which are related to Argonaute, to suppress the expression and movement of transposons in the germ cells of animals. The term Piwi stands for "*P* Element *I*nduced *Wi*mpy testis," which refers to infertility in *Drosophila* associated with mutations in genes that encode Piwi

proteins. Although the mechanism of transposon silencing by piRNAs is not fully understood, we know that it includes the degradation of mRNA transcribed from transposons, changes in chromatin structure that inhibit the transcription of transposons, and inhibition of the translation of proteins encoded by transposons. Piwi-interacting RNAs are also expressed in somatic tissues, where their function is less well understood; evidence suggests that they play a role in transposon silencing, stem-cell maintenance, tissue regeneration, and epigenetic processes.

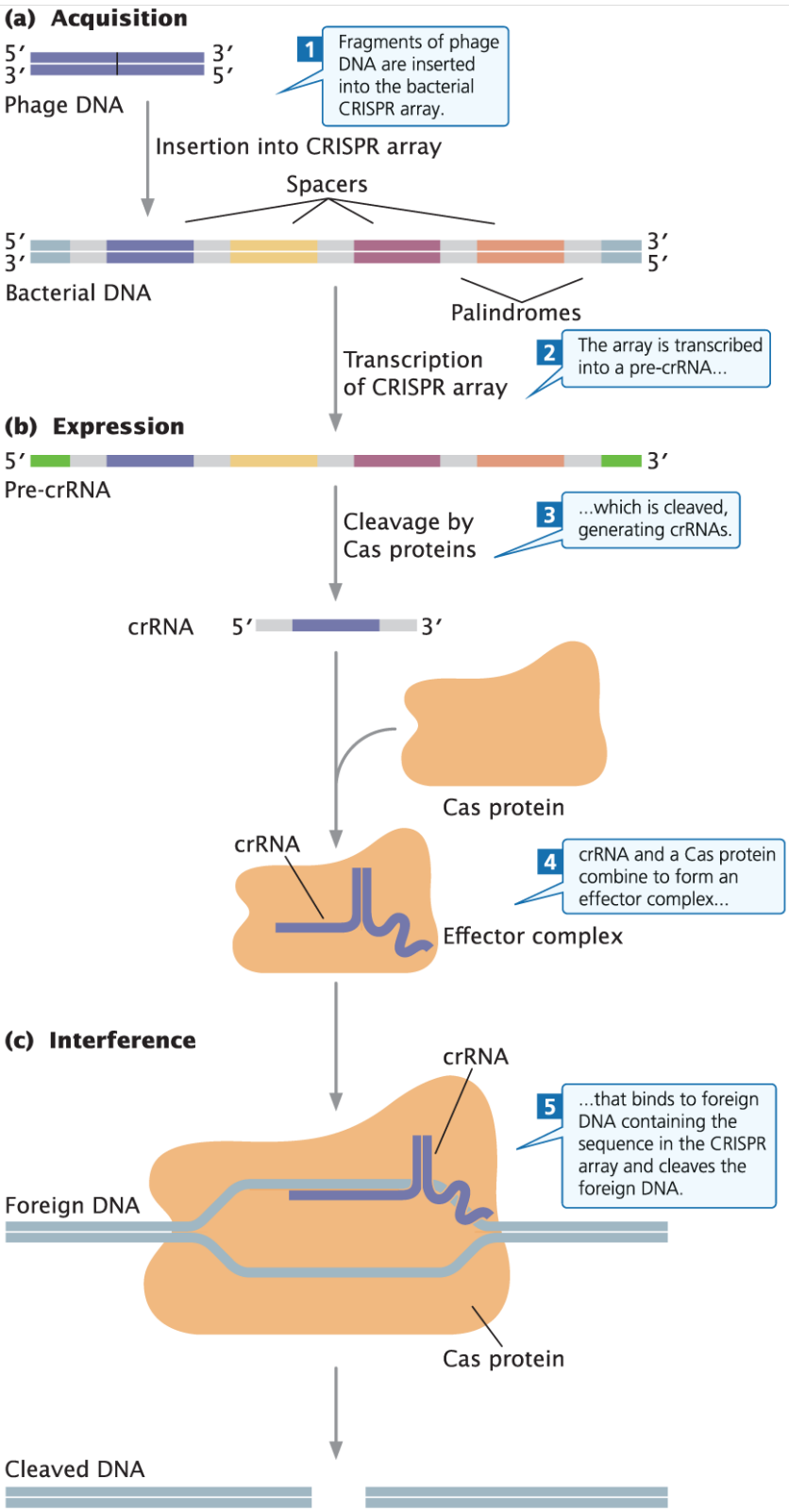
CRISPR RNA

After the discovery of small RNAs in eukaryotes, similar small RNAs, called CRISPR RNAs (crRNAs), were discovered in prokaryotes. CRISPR RNAs are encoded by DNA sequences found in bacterial and archaeal genomes termed *c*lustered *r*egularly *i*nterspaced *s*hort *p*alindromic *r*epeats. Palindromic sequences are sequences that read the same forward and backward on two complementary DNA strands (see Chapter 4). A CRISPR array consists of a series of such palindromic sequences, separated by spacers that are derived from the DNA of foreign bacteriophage or plasmid genomes. For example, in the bacterium *Pseudomonas aeruginosa*, CRISPR palindromic repeats are 28 bp in length, separated by 32 bp spacers.

CRISPR RNAs combine with Cas (CRISPR-associated) proteins to provide defense against the invasion of specific foreign DNA

molecules, such as DNA originating from bacteriophages and plasmids (see Chapter 9). Because they target specific DNA molecules, CRISPR-Cas systems have been compared to the immune systems of vertebrates. CRISPR-Cas systems are widespread in prokaryotes, occurring in 50% of bacterial species and 90% of archaea.

The action of a CRISPR-Cas system takes place in three stages: acquisition, expression, and interference. In acquisition, which is still incompletely understood, foreign DNA from a bacteriophage or plasmid that enters the cell is identified, processed, and inserted into the CRISPR array as a new spacer between the palindromic repeats (**Figure 14.23a**). The spacer DNA then serves as a memory of this foreign DNA.



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14.23 CRISPR RNAs defend prokaryotic cells against invasion by foreign DNA, such as DNA from bacteriophages and plasmids.

Description

Part a at the top shows acquisition where a phage D N A double strand is present. Pieces of phage D N A are inserted into a C R I S P R array below. A caption reads, fragments of phage D N A are inserted into the bacterial C R I S P R array. The phage D N A is inserted near the 5 prime end of a stretch of D N A that contains spacers and palindrome sequences. A caption reads, the array is transcribed into a pre-c r R N A. Transcription of C R I S P R array occurs below. Part b below shows expression. Transcription leads to pre c r R N A sequence. Cleavage by C a s protein occurs and a c r R N A containing phage R N A is produced below. A caption reads, which is cleaved, generating c r R N A's. C A S protein enters the process and an effector complex with c r R N A is formed. A caption reads, c r R N A and a C a s protein combine to form an effector complex. The part c below shows interference. The effector complex bind to a foreign D N A and replication bubble is formed inside the effector complex. The c r R N A bonds to the upper strand of D N A and eventually, the D N A is cleaved into two fragments below. A caption reads, that binds to foreign D N A containing the sequence in the C R I S P R array and cleaves the foreign D N A.

In the second stage—expression—the CRISPR array is transcribed into a long CRISPR precursor RNA (Figure 14.23b), which is then cleaved by Cas proteins and processed into crRNAs, each of which contains one spacer sequence that is homologous to the foreign DNA. The crRNA then combines with a Cas protein to form an effector complex.

The final stage (interference) occurs when foreign DNA from the same bacteriophage or plasmid enters the cell again. The effector complex binds to the foreign DNA through base paring between the

crRNA and the foreign DNA sequence. The Cas protein then cleaves the foreign DNA, rendering it nonfunctional (Figure 14.23c). In this way, crRNAs serve as an adaptive RNA defense system against foreign invaders.

CONCEPTS

Piwi-interacting RNAs are primarily found in the germ cells of animals and inhibit transposons. CRISPR RNAs are found in prokaryotes, where they function in defense against foreign DNA.

14.6 Long Noncoding RNAs Regulate Gene Expression

For many years, our knowledge of RNA was limited to those molecules that play a central role in the synthesis of proteins: mRNAs, tRNAs, and rRNAs. Later, small nuclear RNAs that participate in the posttranscriptional processing of RNA (snRNAs and snoRNAs) were added to the list. Starting in the late 1990s, geneticists began to recognize that numerous small RNAs (siRNAs, miRNAs, piRNAs, and crRNAs) were also abundant and fundamentally important to cell function. More recently, it has become apparent that much of the eukaryotic genome is transcribed—although only about 1% of the human genome, for example, directly encodes proteins, the majority of DNA nucleotides are transcribed, producing many long RNA molecules that do not encode proteins. Called **long noncoding RNAs (lncRNAs)**, these RNAs are typically over 200 nucleotides in length and can be as many as 100,000 nucleotides long. They lack an open reading frame—a sequence with a start and a stop codon that is translated into a protein. Thousands of lncRNAs have been discovered. It is estimated that the human genome generates more than 20,000 different lncRNAs. The DNA sequences that encode them, along with other DNA of unknown function, have been called "the dark matter of the genome."

There is currently much debate about whether most lncRNAs are functionally important or simply represent "transcriptional noise"—the products of frequent, imprecise transcription. Although the function of many lncRNAs is still unclear, there is increasing evidence that at least some play a role in controlling gene expression. Some lncRNAs interact with proteins that regulate transcription. For example, an lncRNA called lincRNA-p21 interacts with a protein called p53, a transcription factor that activates numerous genes, including genes involved in control of the cell cycle, cell death, and other cellular functions. By repressing p53, lincRNA-p21 affects the transcription of hundreds of genes. Other lncRNAs modify chromatin structure, which also regulates transcription (see Chapter 17).

Some lncRNAs have binding sites for proteins or miRNAs that interact with mRNAs; in these cases, the lncRNAs serve as decoys for their attachment, thereby limiting the numbers of miRNAs or proteins that are available to bind to mRNAs. For example, an lncRNA called NORAD (for *no*ncoding *R*NA *a*ctivated by DNA *d*amage), which is present in hundreds of copies in most mammalian cells, is important in maintaining chromosome stability. NORAD has multiple binding sites for PUMILIO proteins, which bind to and repress the stability and translation of mRNAs involved in mitosis, DNA repair, and DNA replication. The NORAD lncRNAs normally sequester PUMILIO proteins so that they are not available to bind to mRNA and repress these processes. Researchers

have found that inactivation of NORAD lncRNA leads to an increase in PUMILIO proteins, causing chromosome instability and aneuploidy. Thus, the NORAD lncRNA plays an essential role in maintaining chromosome stability in mammalian cells by serving as a decoy for PUMILIO proteins.

One of the best-studied lncRNAs is *Xist* RNA, which plays a central role in dosage compensation in mammalian cells (described in Chapter 4). To balance the expression of autosomal genes and X-linked genes in males (with one X chromosome) and females (with two X chromosomes), one of the X chromosomes in each mammalian female cell is inactivated. Which X chromosome is inactivated is random and is determined early in development; once inactivated, this chromosome remains inactive through multiple rounds of cell division. *Xist* RNA is transcribed only from the X chromosome destined to become inactive; the *Xist* RNA coats that chromosome and recruits proteins that methylate histones in the chromatin. Methylation of the chromatin inhibits the transcription of genes on the inactive X chromosome. At least two additional lncRNAs act to regulate the expression of *Xist* RNA.

Still other lncRNAs are complementary to mRNA sequences and function by base pairing with those mRNAs and preventing their translation or splicing. Evidence suggests that some lncRNAs bring about genomic imprinting (see Chapters 4 and 21). Imprinting occurs when a gene is expressed differently depending on whether

it is inherited from the male or the female parent. Many clusters of imprinted genes contain sequences that encode lncRNAs, and evidence suggests that some imprinted genes are controlled by lncRNAs.

Noncoding RNAs called **enhancer RNAs (eRNAs)** are transcribed from enhancer sequences and may play a role in regulating the expression of protein-encoding genes, perhaps by facilitating the looping of DNA between enhancers and promoters (see Chapter 13). Another recently discovered group of noncoding RNAs consists of **circular RNAs (circRNAs)**. Large numbers of these RNAs accumulate in cells, probably because their circular nature makes them resistant to the action of enzymes that normally degrade linear RNA molecules at their 5′ or 3′ ends. Many of these circular noncoding RNAs are thought to arise through errors in splicing in which, for example, the 5′ end of an exon is erroneously joined to the 3′ end of the same exon. A few circRNAs contain numerous binding sites for miRNAs. Like the lncRNAs mentioned earlier, these RNAs can serve as decoys for miRNAs, preventing the miRNAs from attaching to and inhibiting the translation of mRNAs. Other circRNAs contain sequences that code for proteins and appear to be translated. However, the function of most circRNAs, if they indeed have a function, is unknown.

CONCEPTS

Long noncoding RNAs are long RNA molecules that do not encode proteins. Evidence increasingly suggests that many of these molecules function in the control of gene expression. Enhancer RNAs are transcribed from enhancers and also play a role in control of gene expression. Circular noncoding RNAs, which are numerous in some cells, may serve as decoys for miRNAs.

Chapter 14 Review

CHAPTER SUMMARY

Section 14.1 Learning Objective: Be able to discuss how the concept of a gene changed as geneticists learned more about the molecular nature of genetic information.

- A gene is often defined as a sequence of DNA nucleotides that is transcribed into a single RNA molecule, although the widespread occurrence and complexity of transcription makes this definition problematic.
- Introns—noncoding sequences that interrupt the coding sequences (exons) of genes—are common in eukaryotic cells but rare in bacterial cells.

Section 14.2 Learning Objective: Be able to outline the different ways that mRNAs are modified in eukaryotic cells.

- An mRNA molecule has three primary regions: a $5'$ untranslated region, a protein-coding region, and a $3'$ untranslated region.
- Bacterial mRNA is translated immediately after transcription and undergoes little processing. The pre-mRNA of a eukaryotic protein-encoding gene is extensively processed: a modified guanine nucleotide and methyl groups, collectively termed the cap, are added to the $5'$ end of pre-mRNA; the $3'$ end is cleaved

and a poly(A) tail is added; internal nucleotides are modified; and introns are removed.
- Introns are removed from pre-mRNA within a structure called the spliceosome, which is composed of several small nuclear RNAs and proteins.
- Some introns found in rRNA genes and mitochondrial genes are self-splicing.
- Some pre-mRNAs undergo alternative processing, in which different combinations of exons are spliced together or different 3′ cleavage sites are used.
- Messenger RNAs may be altered by the addition, deletion, or modification of nucleotides in the coding sequence, a process called RNA editing.

Section 14.3 Learning Objective: Be able to describe the structure and processing of tRNAs.

- Transfer RNAs, which attach to amino acids, are short molecules that assume a common secondary structure and contain modified bases.
- Transfer RNAs are processed by cleavage, trimming, and nucleotide addition and base modification.

Section 14.4 Learning Objective: Be able to compare the structures of prokaryotic and eukaryotic ribosomes and describe how rRNA is processed.

- Ribosomes, the sites of protein synthesis, are composed of several ribosomal RNA molecules and numerous proteins.

- Prokaryotic and eukaryotic ribosomes consist of small and large subunits but differ in the number of rRNA molecules and proteins.
- Ribosomal RNA is extensively modified in both prokaryotes and eukaryotes.

Section 14.5 Learning Objective: Be able to describe the types of small RNAs and their functions.

- Small interfering RNAs, microRNAs, Piwi-interacting RNAs, and CRISPR RNAs play important roles in gene silencing and in a number of other biological processes.

Section 14.6 Learning Objective: Be able to describe lncRNAs and their functions.

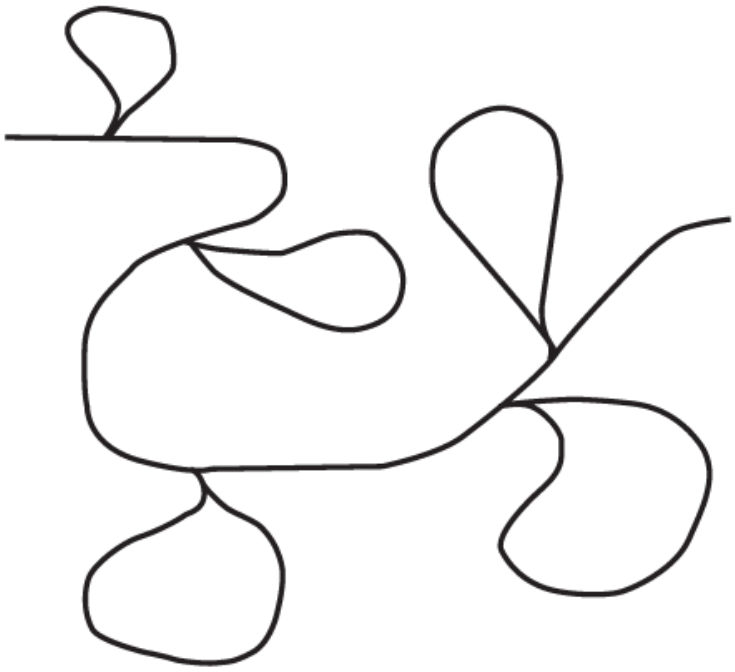
- Long noncoding RNAs are RNA molecules that do not encode proteins. Evidence increasingly suggests that many of these molecules function in the control of gene expression.
- Circular noncoding RNAs are common in many cells; some may serve as decoys for miRNAs.

WORKED PROBLEMS

Problem 1

DNA from a eukaryotic gene was isolated, denatured, and hybridized to the mRNA transcribed from the gene; the hybridized structure

was then observed with an electron microscope. The adjoining diagram shows the structure that was observed.



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a. How many introns and exons are there in this gene? Explain your answer.
b. Identify and label the introns and exons in this hybridized structure.

» Solution Strategy

What information is required in your answer to the problem?

a. The number of introns and exons and how you arrived at your answer.

b. The location of the introns and exons labeled on the figure.

What information is provided to solve the problem?

- The DNA and mRNA are from a eukaryote.
- The DNA was denatured and hybridized to the mRNA.
- A diagram of the hybridized structure.

For help with this problem, review:

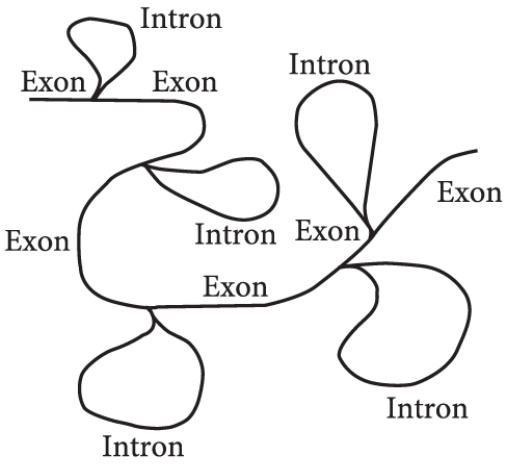
Introns in Section 14.1 and Figure 14.2.

» Solution Steps

a. Each of the loops represents a region in which sequences in the DNA do not have corresponding sequences in the RNA; these regions are introns. There are five loops in the hybridized structure so there must be five introns in the DNA and six exons.

> **Recall:** Introns are noncoding sequences found within eukaryotic genes.

b.



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Description
The drawing shows a wavy line with 3 loops above the line and 2 loops below it. The straight sections of the line between loops are labeled as exons and the loops extending outward from this straight line are labeled as introns.

Hint: The number of introns will be one less than the number of exons.

Problem 2

Draw a typical bacterial mRNA and the gene from which it was transcribed. Identify the 5′ and 3′ ends of the RNA and DNA molecules, as well as the following regions or sequences:

a. Promoter
b. 5′ untranslated region
c. 3′ untranslated region
d. Protein-coding sequence

e. Transcription start site
f. Terminator
g. Shine–Dalgarno sequence
h. Start and stop codons

» Solution Strategy

What information is required in your answer to the problem?

A drawing of an mRNA molecule and the gene from which it is transcribed. The 5′ and 3′ ends of the mRNA and DNA molecules. Locations of the listed structures on the drawing.

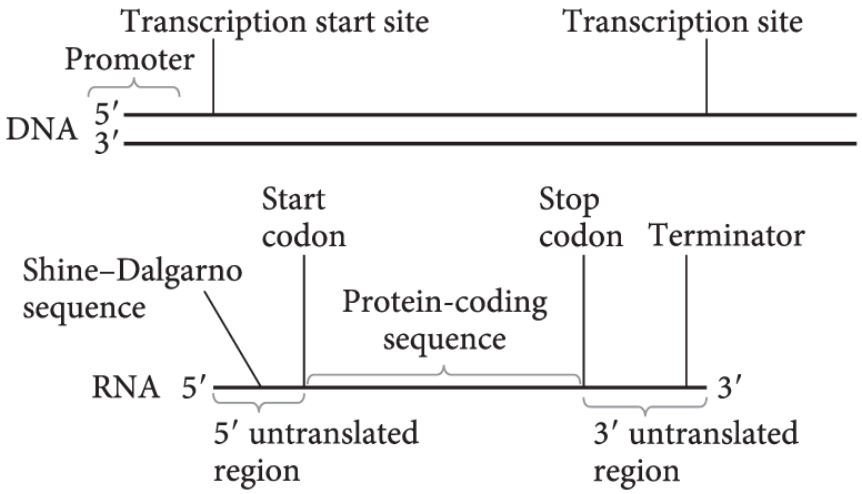
What information is provided to solve the problem?

- The gene is from a bacterium.
- Different parts of the DNA and RNA that are to be labeled.

For help with this problem, review:

The Template in Section 13.2 and The Structure of Messenger RNA in Section 14.2.

» Solution Steps



Description
The double-stranded D N A has the 5 prime to 3 prime strand on top and the 3 prime to 5 prime strand on the bottom. From left to right, the promoter region, transcription start site, and transcription stop site are labeled. A caption reads, Hint: Review the structure of a transcription unit in Figure 13 point 6 and the structure of m R N A in Figure 14 point 5. The drawing shows R N A strand below the D N A strand. From left to right, the regions of the R N A are labeled as follows: 5 prime untranslated region with Shine-Dalgarno sequence, start codon, protein-coding sequence, stop codon, 3 prime untranslated region with a terminator.

Hint: Review the structure of a transcription unit in Figure 13.6 and the structure of mRNA in Figure 14.5.

IMPORTANT TERMS

colinearity
exon
intron
group I intron

group II intron
nuclear pre-mRNA intron
transfer RNA intron
codon
5′ untranslated region (5′ UTR)
Shine–Dalgarno sequence
protein-coding region
3′ untranslated region (3′ UTR)
5′ cap
poly(A) tail
RNA splicing
5′ splice site
3′ splice site
branch point
spliceosome
lariat
trans-splicing
recursive splicing
alternative processing pathway
alternative splicing
multiple 3′ cleavage sites
RNA editing
guide RNA (gRNA)
modified base
tRNA-modifying enzyme
cloverleaf
anticodon
large ribosomal subunit
small ribosomal subunit

RNA interference (RNAi)
RNA-induced silencing complex (RISC)
long noncoding RNA (lncRNA)
enhancer RNA (eRNA)
circular RNA (circRNA)

ANSWERS TO CONCEPT CHECKS

1. When DNA was hybridized to the mRNA transcribed from it, regions of DNA that did not correspond to RNA looped out.
2. Group I introns, group II introns, nuclear pre-mRNA introns, and transfer RNA introns.
3. A protein that adds the 5′ cap is associated with RNA polymerase II, which transcribes pre-mRNAs but is absent from RNA polymerases I and III, which transcribe rRNAs and tRNAs, respectively.
4. b
5. c
6. Guide RNA
7. b
8. d
9. An siRNA or miRNA combines with proteins to form a RISC, which then pairs with mRNA through complementary pairing between bases on the siRNA or miRNA and bases on the mRNA.

Chapter 14 Assessment

COMPREHENSION QUESTIONS

Section 14.1

1. What is the concept of colinearity? In what way is this concept fulfilled in bacterial and eukaryotic cells?

2. What are some characteristics of introns?

3. What are the four basic types of introns? In which organisms are they found?

Section 14.2

4. What are the three primary regions of mRNA sequences in bacterial cells?

5. What is the function of the Shine–Dalgarno consensus sequence?

6. What is the 5′ cap? How is the 5′ cap added to eukaryotic pre-mRNA? What is the function of the 5′ cap?

7. How is the poly(A) tail added to pre-mRNA? What is the purpose of the poly(A) tail?

8. What makes up the spliceosome? What is the function of the spliceosome?

9. Explain the process of pre-mRNA splicing in nuclear genes.

10. Describe two types of alternative processing pathways. How do these pathways lead to the production of multiple proteins from a single gene?

11. What is RNA editing? Explain the role of guide RNAs in RNA editing.

12. Summarize the different types of processing that can take place in pre-mRNA.

Section 14.3

13. What are some of the modifications in tRNA that take place through processing?

Section 14.4

14. Describe the basic structure of ribosomes in bacterial and in eukaryotic cells.

15. Explain how rRNA is processed.

Section 14.5

16. What is the origin of small interfering RNAs, microRNAs, and Piwi-interacting RNAs? What do these RNA molecules do in the

cell?

17. What are some similarities and differences between siRNAs and miRNAs?

18. What role do CRISPR-Cas systems naturally play in bacteria?

19. Outline the three stages of CRISPR-Cas action.

20. Explain how some lncRNAs serve as molecular decoys for RNA-binding proteins and miRNAs.

APPLICATION QUESTIONS AND PROBLEMS

Introduction

21.

What characteristics of the royal family's hemophilia described in the introduction and in the pedigree shown in **Figure 6.8** suggest that their hemophilia was inherited as an X-linked recessive disorder? See Chapter 6 for information about interpreting pedigrees.

Section 14.1

22. *Duchenne muscular dystrophy is caused by a mutation in a gene that comprises 2.5 million base pairs and specifies a protein called dystrophin. However, less than 1% of the gene actually encodes the amino acids in the dystrophin protein. On the basis of what you now know about gene structure and RNA processing in eukaryotic cells, provide a possible explanation for the large size of the dystrophin gene.

23. What would happen in the experiment illustrated in **Figure 14.2** if the DNA and RNA that are mixed together came from very different organisms—for example, a worm and a pig?

24. For the ovalbumin gene shown in **Figure 14.3**, where would the 5′ untranslated region and the 3′ untranslated region be located in the DNA and in the RNA?

25.

For the ovalbumin gene shown in **Figure 14.3**, indicate the locations of the following (see Chapter 13 for information about transcription): (a) transcription start site, (b) template strand, and (c) promoter.

Section 14.2

26. How do the mRNAs of bacterial cells and the pre-mRNAs of eukaryotic cells differ? How do the mature mRNAs of bacterial and eukaryotic cells differ?

27. Are the 5′ untranslated regions (5′ UTR) of eukaryotic mRNAs encoded by sequences in the promoter, exon, or intron of the gene? Explain your answer.

28. *Draw a typical eukaryotic gene and the pre-mRNA and mRNA derived from it. Assume that the gene contains three exons. Identify the following items, and for each item, give a brief description of its function:

    a. 5′ untranslated region

    b. Promoter

    c. AAUAAA consensus sequence

    d. Transcription start site

    e. 3′ untranslated region

    f. Introns

    g. Exons

    h. Poly(A) tail

    i. 5′ cap

29. How would the deletion of the Shine–Dalgarno sequence affect a bacterial mRNA?

30. What would be the most likely effect of moving the AAUAAA consensus sequence shown in **Figure 14.7** 10 nucleotides

upstream?

31. How would the deletion of the following sequences or features most likely affect a eukaryotic pre-mRNA?

    a. AAUAAA consensus sequence

    b. 5′ cap

    c. Poly(A) tail

32. Suppose that a mutation occurs in the middle of a large intron of a gene encoding a protein. What would be the most likely effect of the mutation on the amino acid sequence of that protein? Explain your answer.

33. *A geneticist induces a mutation in a cell line growing in the laboratory. The mutation occurs in a gene that encodes a protein that participates in the cleavage and polyadenylation of eukaryotic mRNA. What will be the immediate effect of this mutation on RNA molecules in the cultured cells?

34. A geneticist induces a mutation in a cell line growing in the laboratory. The mutation occurs in a gene that encodes a protein that binds to the poly(A) tail of eukaryotic mRNA. What will be the immediate effect of this mutation in the cultured cells?

35. A geneticist isolates a gene that contains eight exons. He then isolates the mature mRNA produced by this gene. After making the DNA single stranded, he mixes the single-stranded DNA

with the mRNA. Some of the single-stranded DNA hybridizes (pairs) with the complementary mRNA. Draw a picture of what the DNA–RNA hybrids will look like under the electron microscope.

36. A geneticist discovers that two different proteins are encoded by the same gene. One protein has 56 amino acids, and the other has 82 amino acids. Provide a possible explanation for how the same gene can encode both of these proteins.

37. Suppose that a 20 bp deletion occurs in the middle of exon 2 of the gene depicted in **Figure 14.12a**. What will be the likely effect of this deletion in the proteins produced by alternative splicing?

38. *Explain how each of the following processes complicates the concept of colinearity.

    a. Trans-splicing

    b. Alternative splicing

    c. RNA editing

39. Researchers studying certain transposable elements (see Chapter 18) have found that these transposons are more likely to occur in introns than in exons. Provide a possible explanation for why transposable elements are more likely to occur in introns.

Section 14.5

40. RNA interference may be triggered when inverted repeats are transcribed into an RNA molecule that then folds to form double-stranded RNA. Write out a sequence of inverted repeats within an RNA molecule. Using a diagram, show how the RNA with the inverted repeats can fold to form double-stranded RNA.

41.

In the early 1990s, Carolyn Napoli and her colleagues were working on petunias, attempting to genetically engineer a variety with dark purple petals by introducing numerous copies of a gene that encodes purple pigment in the flower petals (C. Napoli, C. Lemieux, and R. Jorgensen. 1990. *Plant Cell* 2:279–289). Their thinking was that extra copies of the gene would cause more purple pigment to be produced and would result in a petunia with an even darker hue of purple. However, much to their surprise, many of the plants carrying extra copies of the purple gene were completely white or had only patches of color. Molecular analysis revealed that the amount of mRNA produced by the purple gene was reduced 50-fold in the engineered plants compared with wild-type plants. Somehow, the introduction of extra copies of the purple gene silenced both the introduced copies and the plant's own purple genes. Provide a possible explanation for how the introduction of numerous copies of the purple gene silenced all copies of the purple gene.

roger ashford/Alamy

White petunia.

CHALLENGE QUESTIONS

Section 14.2

42. DATA ANALYSIS Alternative splicing takes place in more than 95% of the human protein-encoding genes with multiple exons. Researchers have found that how a pre-mRNA is spliced is affected by the pre-mRNA's promoter sequence (D. Auboeuf et al. 2002. *Science* 298:416–419). In addition, factors that affect the rate of elongation by the RNA polymerase during transcription affect the type of splicing that takes place. These findings suggest that the process of transcription affects splicing. Propose one or more mechanisms that would explain how transcription might affect alternative splicing.

43. DATA ANALYSIS Duchenne muscular dystrophy (DMD) is an X-linked recessive genetic disease caused by mutations in the gene that encodes dystrophin, a large protein that plays an important role in the development of normal muscle fibers. The dystrophin gene is immense, spanning 2.5 million base pairs, and includes 79 exons and 78 introns. Many of the mutations that cause DMD produce premature stop codons, which bring protein synthesis to a halt, resulting in a greatly shortened and nonfunctional form of dystrophin. Some geneticists have proposed treating DMD patients by causing the spliceosome to skip the exon containing the stop codon. Exon skipping would produce a protein that is somewhat shortened (because an exon is skipped and some amino acids are missing) but might still result in a protein that had some function (A. Goyenvalle et al. 2004. *Science* 306:1796–1799). Propose a possible mechanism to bring about exon skipping for the treatment of DMD.

44. In eukaryotic cells, a poly(A) tail is normally added to pre-mRNA but not to rRNA or tRNA. With the use of recombinant DNA techniques, a protein-encoding gene (which is normally transcribed by RNA polymerase II) can be connected to a promoter for RNA polymerase I. This hybrid gene is subsequently transcribed by RNA polymerase I, and the appropriate pre-mRNA is produced, but this pre-mRNA is not cleaved at the 3′ end, and a poly(A) tail is not added. Propose a mechanism to explain how the type of promoter found at the 5′ end of a gene can affect whether a poly(A) tail is added to the 3′ end.

45. SR proteins are essential to proper spliceosome assembly and are known to take part in the regulation of alternative splicing. Surprisingly, the role of SR proteins in splice-site selection and alternative splicing is affected by the promoter used for the transcription of the pre-mRNA. For example, through genetic engineering, RNA polymerase II promoters that have somewhat different sequences can be created. When pre-mRNAs with exactly the same sequences are transcribed from two different RNA polymerase II promoters that differ slightly in sequence, the promoter used can affect how the pre-mRNA is spliced. Propose a mechanism by which the DNA sequence of an RNA polymerase II promoter could affect alternative splicing of pre-mRNA.

🗣️ ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 14.1

1. Eukaryotic genes are often interrupted by noncoding introns. What might be some possible reasons that organisms have evolved introns? And why might other organisms lose introns?

2. Suppose that you are at a party on Friday night, relaxing after your big genetics exam. Someone comes up to you and, hearing that you just finished your genetics exam, says, “What exactly

is a gene?" How would you respond? What are the strengths and weaknesses of your definition of a gene?

Section 14.2

3. Many human genetic diseases are caused by mutations that occur at splice sites. Propose some ways that mutations at the 5′ splice site, 3′ splice site, and branch point might disrupt splicing and alter the phenotype.

Section 14.5

4. Small RNA molecules are involved in numerous genetic processes, including replication, translation, mRNA processing and degradation, inhibition of translation, chromatin modification, and protection against viruses and transposable elements. However, small DNA molecules have little or no role in these functions. Why has RNA and not DNA evolved to carry out these functions?

5. The number of microRNAs encoded by the genome varies widely among organisms: some species have many miRNA genes, and other species have relatively few. Researchers have determined the number of miRNA genes possessed by different species and have observed a strong positive correlation between the number of miRNA genes on a chromosome and the number of protein-encoding genes on that chromosome. In other words, chromosomes with more protein-encoding genes have more miRNA genes. However, the number of miRNA

genes found on a chromosome is not correlated with chromosome length. Propose a possible explanation for these observations. (Hint: Think about where sequences that code for miRNAs are found.)

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Create a concept map that outlines the inheritance of hemophilia in the royal families of Europe and how the molecular nature of Alexei's hemophilia was discovered. Include at least the following concepts in your map: royal family, hemophilia, Alexei, Alexandra, Queen Victoria, X-linked inheritance, coagulation factor VIII, coagulation factor IX, exons, introns, RNA splicing.

Section 14.2

2. Draw a concept map that summarizes the different types of processing that occur in mRNA of eukaryotes and the functions of these modifications.
3. Draw a concept map that shows the relationships between different concepts and terms involved in RNA splicing, including the following: pre-mRNA, mRNA, RNA splicing,

alternative splicing, exons, introns, 5′ splice site, 3′ splice site, branch point, spliceosome, lariat, snRNA, snRNP, proteins, minor splicing.

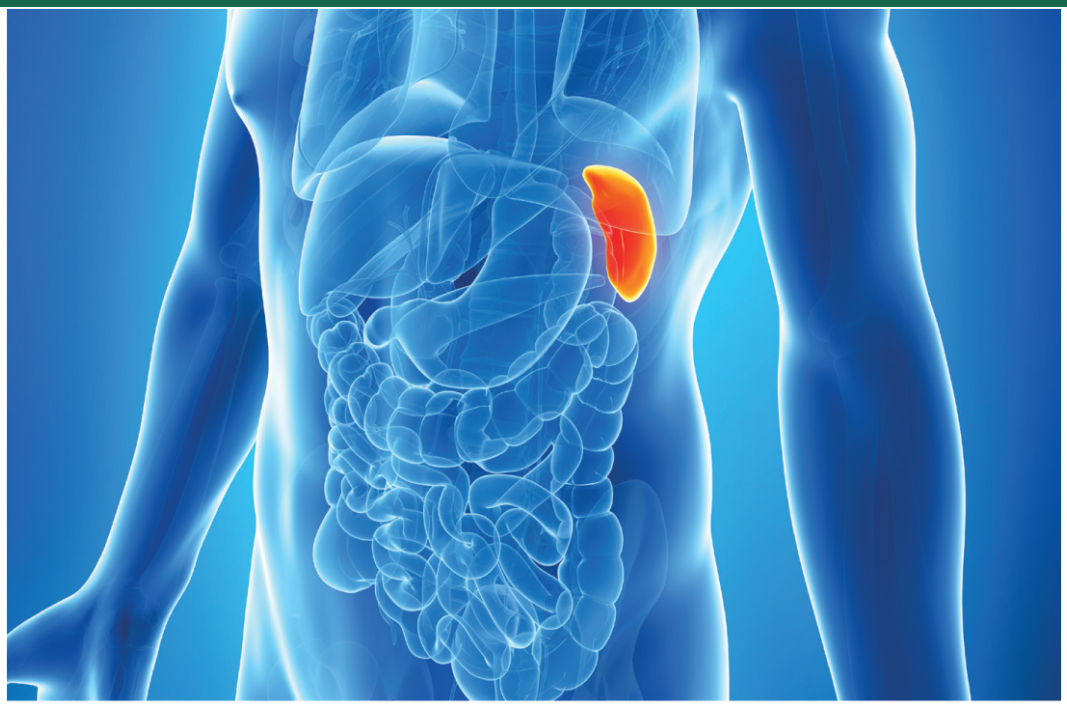
Sections 14.2–14.6

4. Show the relationships between different types of RNAs and their functions in a concept map that includes the following types of RNA along with their functions: DNA, pre-mRNA, mRNA, snRNA, snoRNA, miRNA, siRNA, guide RNA, crRNA, tRNA, rRNA, precursor rRNA, precursor tRNA, piRNA, crRNA, translation, RNA editing, control of gene expression, CRISPR-Cas immunity.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 15

The Genetic Code and Translation



Sebastian Kaulitzki/Shutterstock.

The spleen, an organ found in the upper abdomen, plays an important role in defense against infection. Isolated congenital asplenia is an autosomal dominant condition in which children are born without a spleen.

A Child Without a Spleen

The spleen is an underappreciated organ. Brownish in color and weighing about a third of a pound, it sits in the left upper-part of

your abdomen, storing blood and filtering out bacteria and old blood cells. The spleen is underappreciated because it's widely believed that you can live without a spleen. Indeed, many people who lose their spleen to automobile accidents and other trauma do survive, although they are at increased risk of infection. But a young child without a spleen is in serious trouble. A small group of children are born without spleens; these kids are highly susceptible to life-threatening bacterial infections, and many die in childhood. This rare disorder, known as isolated congenital asplenia (ICA), is inherited as an autosomal dominant trait.

Except for the absence of a spleen, children with ICA are unaffected. But their immune function is severely compromised. When infected with bacteria that the immune system normally eliminates, these children develop raging infections that quickly spread throughout the body. Even when treated with modern antibiotics, they often die.

In 2013, an international team led by scientists from Rockefeller University discovered the genetic cause of ICA. Using the power of DNA sequencing, they examined all the coding DNA of 23 individuals with ICA and compared their DNA sequences with those of 508 individuals with normal spleens. Statistical analysis pointed to differences in one particular gene that was associated with ICA, a gene-encoding ribosomal protein SA (RPSA). The RPSA protein is one of the 33 proteins that make up the small subunit of the ribosome, the molecular complex responsible for protein synthesis.

How a defect in the *RPSA* gene results in the absence of a spleen is not known. Diseases such as ICA, which result from defective ribosomes, are referred to as ribosomopathies.

Many, but not all, individuals with ICA have mutations in *RPSA*, indicating that other genes may also be involved in the disorder. The researchers found several different types of mutations in *RPSA* associated with ICA: some caused premature stop codons, halting translation before a functional protein could be made; one was a frameshift mutation, a change that alters the way the mRNA sequence is read during translation; and others changed the amino acid sequence of the RPSA protein.

One interesting but unanswered question is why a defect in *RPSA* affects only the spleen. Inherited mutations in *RPSA* occur in every cell of the body, and protein synthesis—carried out by ribosomes—is essential for numerous life processes, yet these mutations affect only the development of the spleen. Why aren't other organs altered? Why aren't numerous physiological functions affected? Scientists are still studying these important questions.

THINK-PAIR-SHARE

- Propose some possible reasons why mutations in the *RPSA* gene affect a specific organ like the spleen and not other tissues where ribosomes carry out translation.
- What are some possible reasons that researchers might be interested in identifying the gene that causes a genetic disease such as ICA? In other words, what benefits might result from this research?

Isolated congenital asplenia illustrates the extreme importance of translation, the process of protein synthesis, which is the focus of this chapter. We begin by examining the molecular relation between genotype and phenotype. Next, we study the genetic code —the instructions that specify the amino acid sequence of a protein —and then examine the mechanism of translation. Our primary focus is protein synthesis in bacterial cells, but we also examine some features of this process in eukaryotic cells. At the end of the chapter, we look at some additional aspects of protein synthesis.

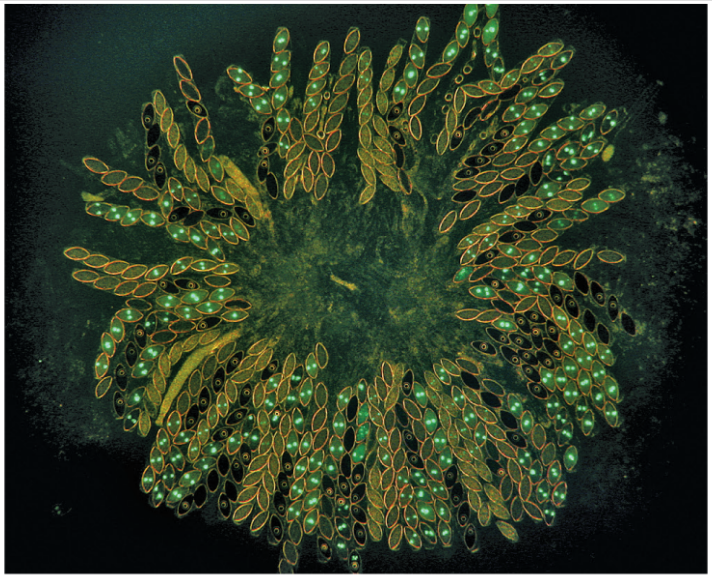
15.1 Many Genes Encode Proteins

The first person to suggest the existence of a relation between genotype and proteins was English physician Archibald Garrod. In 1908, Garrod correctly proposed that genes encode enzymes, but unfortunately, his theory made little impression on his contemporaries. Not until the 1940s, when George Beadle and Edward Tatum examined the genetic basis of biochemical pathways in the bread mold *Neurospora,* did the relation between genes and proteins become widely accepted. Beadle and Tatum's work helped define the relation between genotype and phenotype by leading to the one gene, one enzyme hypothesis: the idea that each gene encodes a separate enzyme.

THINK-PAIR-SHARE Question 1

The One Gene, One Enzyme Hypothesis

Beadle and Tatum used *Neurospora* to study the biochemical results of mutations. *Neurospora* is easy to cultivate in the laboratory, and the main vegetative part of the fungus is haploid, which allows the effects of otherwise recessive mutations to be easily observed (Figure 15.1).



Namboori B. Raju, Stanford University.

15.1 Beadle and Tatum used the fungus *Neurospora*, which has a complex life cycle, to work out the relation of genes to proteins.

Wild-type *Neurospora* grows on a minimal medium, which contains only inorganic salts, nitrogen, a carbon source such as sucrose, and the vitamin biotin. The fungus can synthesize all the biological molecules that it needs from these basic compounds. However, mutations may arise that disrupt fungal growth by destroying the fungus's ability to synthesize one or more essential biological molecules. These nutritionally deficient mutants, termed *auxotrophs* (see Chapter 9), cannot grow on a minimal medium, but they can grow on a medium that contains the substance that they are no longer able to synthesize.

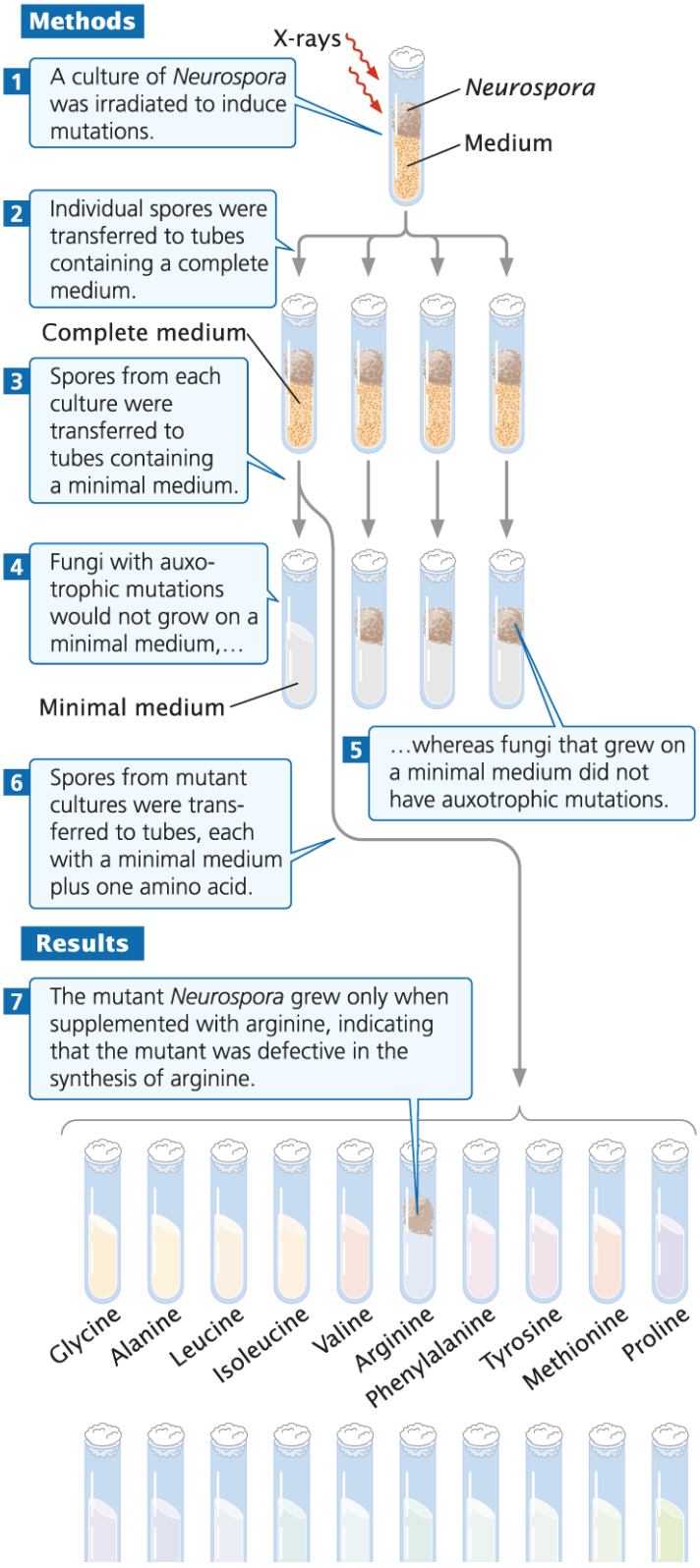
Beadle and Tatum first irradiated spores of *Neurospora* to induce mutations (**Figure 15.2**). Then they placed the spores in different culture tubes with a complete medium (a medium containing all the

biological substances needed for growth). These spores grew into fungi and produced spores by mitosis. Next, they transferred spores from each culture to tubes containing a minimal medium. Fungi with auxotrophic mutations did not grow on the minimal medium, which allowed Beadle and Tatum to identify cultures that possessed mutations.

Experiment

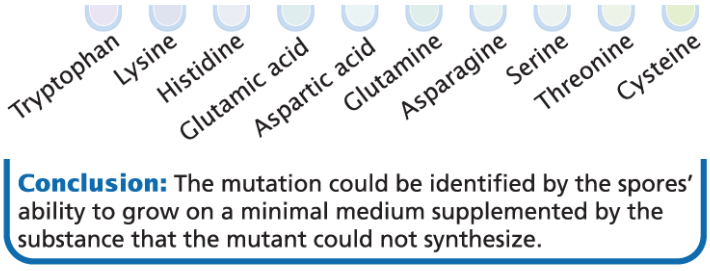
Question: How can auxotrophic mutations be isolated and identified?

Methods



Results

7 The mutant *Neurospora* grew only when supplemented with arginine, indicating that the mutant was defective in the synthesis of arginine.



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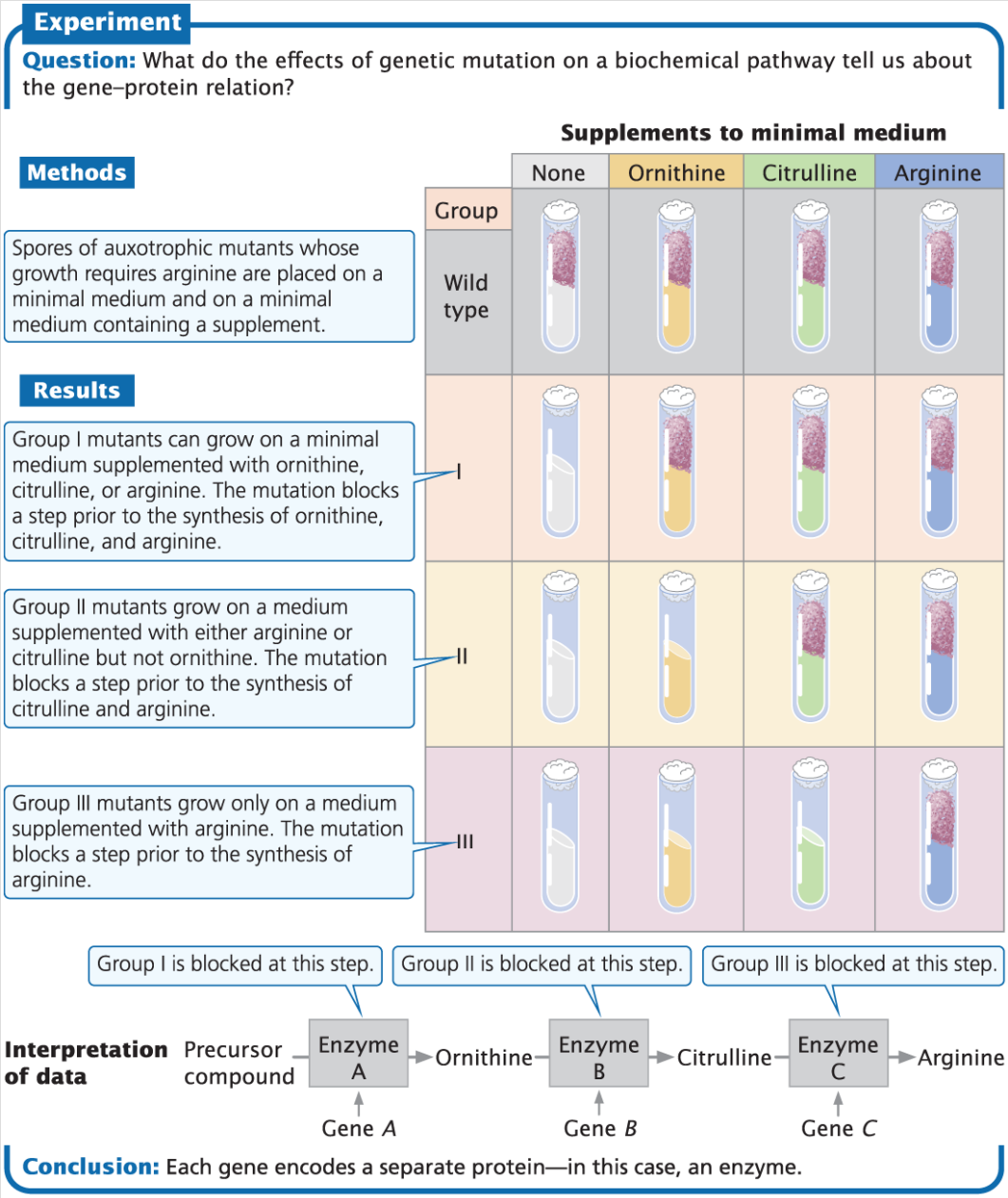
15.2 Beadle and Tatum developed a method for isolating auxotrophic mutants in *Neurospora*.

Description

The question in this experiment is: How can auxotrophic mutations be isolated and identified? The next part below shows methods. The illustration shows a test tube with Neurospora irradiated by X-rays to induce mutations. Below this , 4 tubes have a complete medium. A caption reads, a culture of Neurospora was irradiated to induce mutations. Individual spores were transferred to tubes containing complete medium. In the next step below, spores from those tubes are transferred to 4 tubes with minimal medium. 1 of the 4 tubes does not contain growing Neurospora. A caption reads, spores from each culture were transferred to tubes containing minimal medium. Fungi with auxotrophic mutations would not grow on minimal medium whereas fungi that grew on minimal medium did not have auxotrophic mutations. Three of the four tubes contain growing Neurospora, and have a brown substance on top of the liquid in the tube. In the next step below, the spores from the complete medium tube that could not grow in minimal medium are transferred to 20 tubes, each containing a different amino acid. The amino acids are as follows: Glycine, Alanine, Leucine, Isoleucine, Valine, Methionine, Tyrosine, Arginine, Phenylalanine Proline, Tryptophan, Lysine, Histidine, Glutamic acid, Aspartic acid, Glutamine, Asparagine, Serine, Threonine, and Cysteine. The results section below shows that the mutant Neurospora only grows in the tube with arginine. A caption reads, the mutant Neurospora grew only when supplemented with arginine, indicating that the mutant was defective in the synthesis of arginine. A caption below reads, conclusion: the mutation could be identified by the spores' ability to grow in minimal medium supplemented by the substance that the mutant could not synthesize.

Once they had determined that a particular culture had an auxotrophic mutation, Beadle and Tatum set out to determine the specific effect of the mutation. They transferred spores of each mutant strain from the complete medium to a series of tubes (see Figure 15.2), each of which contained a minimal medium plus one of a variety of essential biological molecules, such as an amino acid. If the spores in a tube grew, Beadle and Tatum were able to identify the added substance as the biological molecule whose synthesis had been affected by the mutation. For example, an auxotrophic mutant that would grow only on a minimal medium to which arginine had been added must have possessed a mutation that disrupts the synthesis of arginine.

Adrian Srb and Norman H. Horowitz patiently applied this procedure to genetically dissect the multistep biochemical pathway of arginine synthesis (**Figure 15.3**). They first isolated a series of auxotrophic mutants whose growth required arginine. Then they tested these mutants for their ability to grow on a minimal medium supplemented with three compounds: ornithine, citrulline, and arginine. From the results, they were able to place the mutants into three groups based on which substances allowed growth (**Table 15.1**).



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15.3 Method used to determine the relation between genes and enzymes in *Neurospora*. The biochemical pathway shown here leads to the synthesis of arginine in *Neurospora*. Steps in the pathway are catalyzed by enzymes affected by mutations.

Description

The illustration shows a question at the top that reads: what do the effects of genetic mutation on a biochemical pathway tell us about the gene-protein relationship? The methods below are shown in the form of a table. The columns show supplements to minimal medium and are labeled as follows: none, ornithine, citrulline, and arginine. The rows are labeled wild type, group 1, group 2, and group 3. All cells in the row labeled wild type contain tubes with growing fungi. A caption reads, spores of auxotrophic mutants whose growth requires arginine are placed on minimal medium and on minimal medium containing a supplement. The results below show the following: In row one, only the tube in column none is not growing. A caption reads, group 1 mutants can grow on minimal medium supplemented with ornithine, citrulline, or arginine. The mutation blocks a step prior to the synthesis of ornithine, citrulline, and arginine. In row 2, the tubes in columns none and ornithine do not contain growing fungus. A caption reads, group 2 mutants grow on medium supplemented with either arginine or citrulline but not ornithine. The mutation blocks a step prior to the synthesis of citrulline and arginine. In row 3, only the tube in the column arginine has growing fungi. A caption reads, group 3 mutants grow only on medium supplemented with arginine. The mutation blocks a step prior to the synthesis of arginine. Below these results is a drawing of the biochemical pathway. The precursor undergoes change to convert into ornithine through enzyme A. Enzyme A is affected by gene A and group 1 is blocked at this step. In the next step, ornithine changes to cirtulline through enzyme B. Enzyme B is affected by gene B and group 2 is blocked at this step. In the next step, citrilline converts to arginine in the presence of enzyme C. Enzyme C is affected by gene C and group 3 is blocked at this step. A caption below reads, conclusion: each gene encodes a separate protein, in this case, an enzyme.

TABLE 15.1 Growth of arginine auxotrophic mutants on a minimal medium with various supplements

Mutant Strain	Ornithine	Citrulline	Arginine
Group I	+	+	+
Group II	−	+	+
Group III	−	−	+

Note: A plus sign (+) indicates growth; a minus sign (−) indicates no growth.

Based on these results, Srb and Horowitz proposed that the biochemical pathway leading to the amino acid arginine has at least three steps:

$$\text{precursor} \xrightarrow{\text{Step 1}} \text{ornithine} \xrightarrow{\text{Step 2}} \text{citrulline} \xrightarrow{\text{Step 3}} \text{arginine}$$

They concluded that the mutations in group I affect step 1 of this pathway, mutations in group II affect step 2, and mutations in group III affect step 3. But how did they know that the order of the compounds in the biochemical pathway was correct?

Notice that if step 1 is blocked by a mutation, then the addition of either ornithine or citrulline allows growth because these compounds can still be converted into arginine (see Figure 15.3). Similarly, if step 2 is blocked, the addition of citrulline allows growth, but the addition of ornithine has no effect. If step 3 is blocked, the spores will grow only if arginine is added to the medium. The underlying principle is that an auxotrophic mutant cannot grow on a compound that comes before a step that is blocked by a mutation in the pathway. The mutant can still synthesize compounds that come after the block, provided the right precursor is supplied.

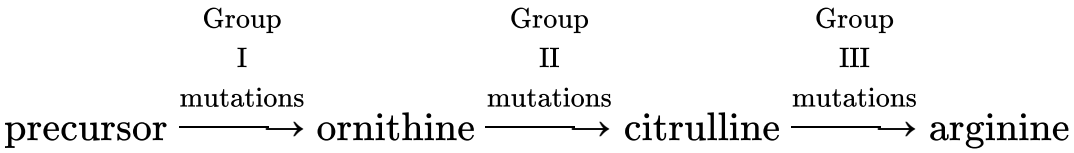
Using this reasoning with the information in Table 15.1, we can see that the addition of arginine to the medium allows all three groups of mutants to grow. Therefore, biochemical steps affected by all the mutants precede the step that results in arginine. The addition of citrulline allows group I and group II mutants to grow, but not group III mutants; therefore, group III mutations must affect a biochemical step that takes place after the production of citrulline but before the production of arginine:

$$\text{citrulline} \xrightarrow[\text{mutations}]{\text{Group III}} \text{arginine}$$

The addition of ornithine allows the growth of group I mutants, but not group II or group III mutants; thus, mutations in groups II and III affect steps that come after the production of ornithine. We've already established that group II mutations affect a step before the production of citrulline; so group II mutations must block the conversion of ornithine into citrulline:

$$\text{ornithine} \xrightarrow[\text{mutations}]{\text{Group II}} \text{citrulline} \xrightarrow[\text{mutations}]{\text{Group III}} \text{arginine}$$

Because group I mutations affect some step before the production of ornithine, we can conclude that they must affect the conversion of some precursor into ornithine. We can now outline the biochemical pathway yielding ornithine, citrulline, and arginine:



Importantly, this procedure does not necessarily detect all steps in a pathway; rather, it detects only the steps that produce the compounds tested.

Using mutations and this type of reasoning, Beadle, Tatum, and others were able to identify the genes that control several biosynthetic pathways in *Neurospora*. They established that each step in a pathway is controlled by a different enzyme, as shown in Figure 15.3 for the arginine pathway. In addition, by conducting genetic crosses and mapping experiments (see Chapter 7), they were able to demonstrate that mutations affecting any one step in a pathway always occurred at the same chromosomal location. Beadle and Tatum reasoned that mutations affecting a particular biochemical step occurred at a single locus that encoded a particular enzyme. This idea became known as the **one gene, one enzyme hypothesis**: genes function by encoding enzymes, and each gene encodes a separate enzyme. Although the genes Beadle and Tatum examined encoded enzymes, many genes encode proteins that are not enzymes, so more generally, their idea was that each gene encodes a protein. When research findings showed that some proteins are composed of more than one polypeptide chain and that different polypeptide chains are encoded by separate genes, this model was

modified to become the one gene, one polypeptide hypothesis. ›

TRY PROBLEM 16

THINK-PAIR-SHARE Question 2

CONCEPTS

Beadle and Tatum's studies of biochemical pathways in the fungus *Neurospora* helped define the relation between genotype and phenotype by establishing the one gene, one enzyme hypothesis, the idea that each gene encodes a separate enzyme. This idea was later modified to the one gene, one polypeptide hypothesis.

✔ CONCEPT CHECK 1

Auxotrophic mutation 103 grows on a minimal medium supplemented with A, B, or C; mutation 106 grows on a medium supplemented with A or C, but not B; and mutation 102 grows only on a medium supplemented with C. What is the order of A, B, and C in the biochemical pathway?

The Structure and Function of Proteins

Proteins are central to all living processes (Figure 15.4). Many proteins are enzymes, the biological catalysts that drive the chemical reactions of the cell; others are structural components, providing scaffolding and support for membranes, filaments, bone,

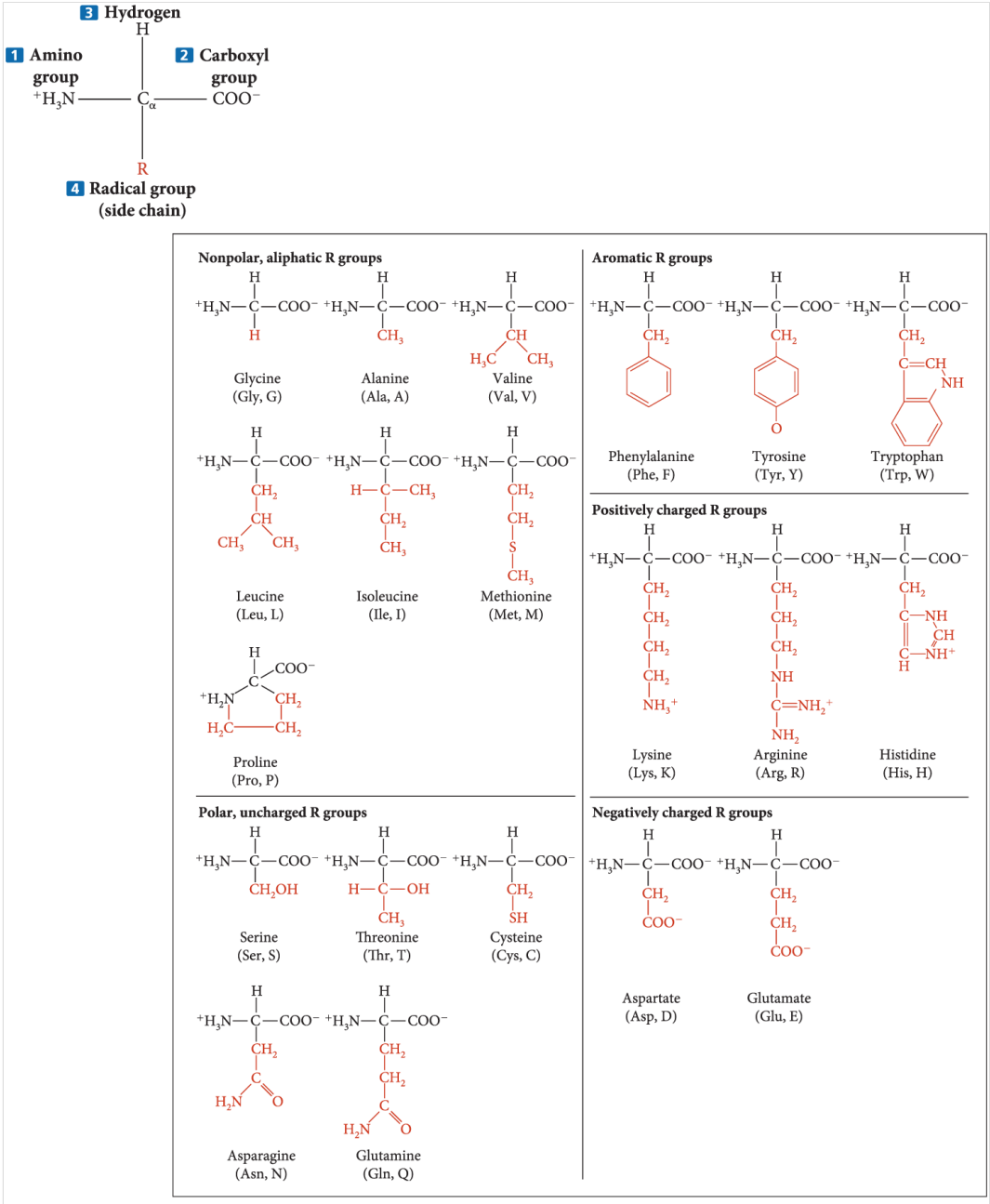
and hair. Some proteins help transport substances; others have a regulatory, communication, or defense function.

Part a: Darwin Dale/Science Source. Part b: Rosemary Calvert/Imagestate/Media Bakery. Part c: REDA&CO/Getty Images.

15.4 Proteins serve a number of biological functions. (a) The light produced by fireflies is the result of a light-producing reaction between luciferin and ATP catalyzed by the enzyme luciferase. (b) The protein fibroin is the major structural component of spiderwebs. (c) Castor beans contain a highly toxic protein called ricin.

AMINO ACIDS

All proteins are polymers composed of **amino acids**, linked end to end. Twenty common amino acids are found in proteins; these amino acids are shown in **Figure 15.5** with both their three-letter and one-letter abbreviations (other amino acids sometimes found in proteins are modified forms of these common amino acids). All of the common amino acids are similar in structure: each consists of a central carbon atom bonded to an amino group, a hydrogen atom, a carboxyl group, and an R (radical) group that differs for each amino acid. The R groups help determine the chemical properties of the amino acids (see Figure 15.5).



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15.5 The 20 common amino acids that make up proteins have similar structures. Each amino acid consists of a central carbon atom (Cα) attached to (1) an amino group (NH_3^+), (2) a carboxyl group (COO^-), (3) a hydrogen atom (H), and (4) a radical group, designated R. In the structures shown here, the parts in black are common to all amino acids and the parts in red are the R groups, which are unique to each amino acid.

Description

The illustration shows a C subscript alpha atom in the center. To its left, is an amino group N H subscript 3 superscript positive. To its right, is a carboxyl group C O O superscript negative. On top of it is a hydrogen atom H, and below is a radical group R (side chain). The R group is shown in red color while all other groups are in black.

The illustration shows 7 nonpolar aliphatic R groups at the top left. These include glycine (G l y, G), alanine (A l a, A), valine (V a l, V), leucine (L e u, L), isoleucine (I l e, I), methionine (M e t, M), and proline (P r o, P). The R group of glycine is hydrogen, the R group of alanine is a methyl group (C H subscript 3), the R group of valine is C subscript 3 H subscript 7 where 3 carbons drawn in the shape of a upside down V. The R group for leucine is C subscript 4 H subscript 9, which looks like valine except with an extra carbon before the upside down V-shaped end. The R group for isoleucine is also C subscript 4 H subscript 9, where the first carbon in the chain has a methyl group attached. The R group of methionine is C subscript 3 H subscript 7 S. The chain has 2 carbons, then sulfur, the ends with a methyl group. The R group of proline is C subscript 3 H subscript 6, and the last carbon in the chain forms a bond with the nitrogen in the amino side group.

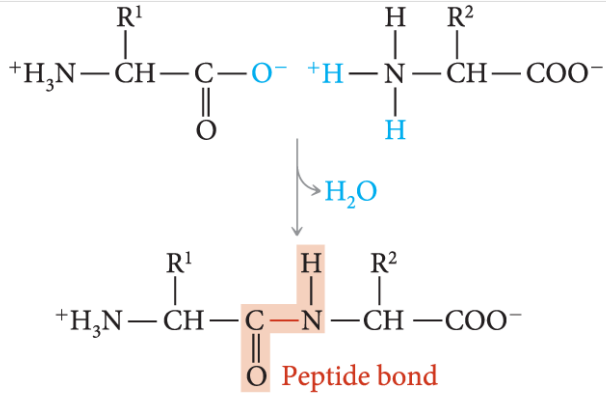
The illustration shows 5 polar, uncharged R groups at the bottom left. These include serine (S e r, S), threonine (T h r, T), cysteine (C y s, C), asparagine (A s n, N), and glutamine (G l n, Q). The R group for serine is C H subscript 2 O H. The R group for threonine is C subscript 2 H subscript 4 O H. The R group for cysteine is C H subscript 2 S H. The R group for asparagine is C H subscript 2 which is further bonded to C O N H subscript 2. The R group of glutamine has two C H subscript 2 groups in a series that are further bonded to C O N H subscript 2.

The illustration shows 3 aromatic R groups at the top right, that include phenylalanine (P h e, F), tyrosine (T r y, Y), and tryptophan (T r p, W). The R group for phenylalanine has one C H subscript 2 group attached to a 6-carbon aromatic ring. The R group of tyrosine is identical to phenylalanine except with an oxygen molecule extending on the end. The R group for tryptophan has a carbon attached to a pentagon structure attached to an aromatic carbon ring (C 9 H subscript 7 N H). The peak in the pentagon is a nitrogen with a hydrogen side chain. The pentagon has 2 double-bonded sides, one of which it shares with the aromatic ring.

The illustration shows positively charged R groups, at the center right, including lysine (L y s, K), arginine (A r g, R), and histidine (H i s, H). The R group for lysine is C subscript 4 H

subscript 8 N H subscript 3 superscript positive. This is an unbranched carbon chain ending with the positively charged amino group. The R group of arginine is C subscript 3 H subscript 6 N H C N subscript 2 H subscript 4 superscript positive. This chain has 3 carbons, a nitrogen, and then a carbon with two N H subscript 2 groups. One of the nitrogens is double-bonded and positively charged. The R group of histidine is C subscript 4 H subscript 5 N subscript 2 H superscript positive. It contains a pentagonal ring with 2 double bonds. One is between 2 carbons and the other is between the nitrogen and carbon at the peak of the pentagon. The double-bonded nitrogen is positively charged. The illustration finally shows negatively charged R groups, at bottom right, that include aspartate (A s p, D) and glutamate (G l u, E). The R group for aspartate is C H subscript 2 C O O superscript negative. The R group for glutamate has two C H subscript 2 groups bonded to C O O superscript negative.

The amino acids in proteins are joined together by **peptide bonds** (**Figure 15.6**) to form **polypeptide** chains; a protein consists of one or more polypeptide chains. Like nucleic acids, polypeptides have polarity under physiological conditions: one end (often called the *amino end*) has a free amino group (NH_3^+), and the other end (the *carboxyl end*) has a free carboxyl group (COO^-). Proteins consist of 50 or more amino acids; some have as many as several thousand.



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15.6 Amino acids are joined together by peptide bonds. A peptide bond (red bar) covalently attaches the carboxyl group of one amino acid to the amino group of another amino acid (pink shading).

Description
Two amino acids are present. The first amino acid is N H 3 superscript positive, C bonded to R 1 and H, C double bond O single bond O superscript negative. O superscript negative is highlighted. The second amino acid is N H 3 superscript positive C bonded to R 2 H, C O O superscript negative. Two of the 3 H bonded to N are highlighted. One of them has a positive charge. A forward arrow shows elimination of H 2 O. The next structure is the combination of the two amino acids removing the highlighted atoms and ions. The structure is as follows:

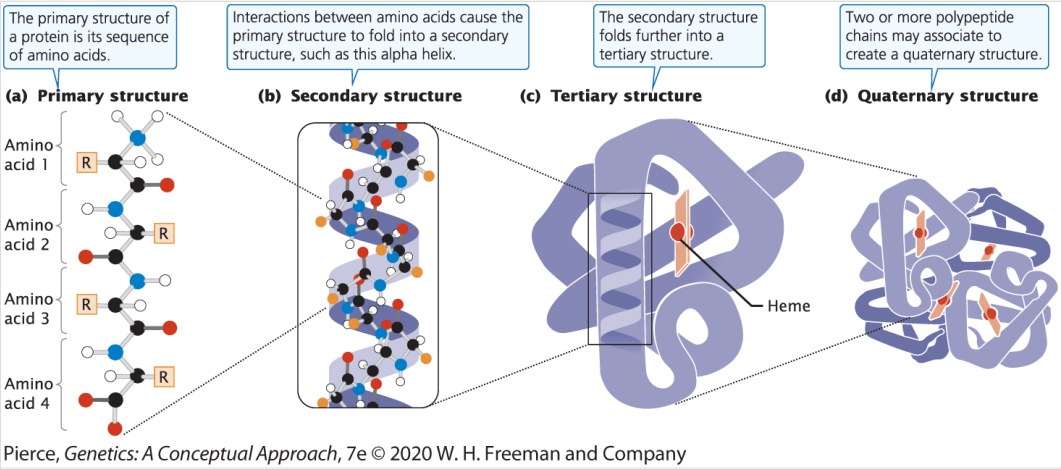
N H 3 single bonded to C H that is further bonded to R 1 on top. The C H is further single bonded to C which is double bonded to an O atom and single bonded to N which is further bonded to H on top and C H on the right. This C H is further bonded to R 2 on top and C O O superscript negative on the right. C O N H at the center is highlighted in the structure.

PROTEIN STRUCTURE

Like that of nucleic acids, the molecular structure of proteins has several levels of organization. The *primary structure* of a protein is its sequence of amino acids (Figure 15.7a). Through interactions between neighboring amino acids, a polypeptide chain folds and twists into a *secondary structure* (Figure 15.7b). Two common secondary structures found in proteins are the beta (β) pleated sheet and the alpha (α) helix. Secondary structures interact and fold further to form a *tertiary structure* (Figure 15.7c), which is the overall, three-dimensional shape of the protein. The secondary and

tertiary structures of a protein are largely determined by the primary structure—the amino acid sequence—of the protein. Finally, some proteins consist of two or more polypeptide chains that associate to produce a *quaternary structure* (**Figure 15.7d**). Many proteins have an additional level of organization defined by domains. A *domain* is a group of amino acids that forms a discrete functional unit within the protein. For example, there are several different types of protein domains that function in DNA binding (see Chapter 16). Gene sequences that encode protein domains are discussed in Chapter 20.

THINK-PAIR-SHARE Question 3



15.7 Proteins have several levels of structural organization.

Description

Part a on the extreme left, with the heading primary structure shows 4 amino acids attached in series to each other. Each amino acid has 4 different molecules along with an R group. The molecules are represented by spheres colored white, black, blue, and red. The R group in amino acids 1 to 4 is attached in alternate pattern. A caption pointing to the heading reads, The primary structure of a protein is its sequence of amino acids.

Part b to its right with the heading secondary structure shows the structure in illustration a as a part of a helix. A caption pointing to the heading reads, Interactions between amino acids cause the primary structure to fold into a secondary structure, such as this alpha helix.

Part c to its right with the heading tertiary structure shows a folded structure having a rectangular body dividing a small red-colored sphere into two hemispheres at its center. The red sphere is labeled

as heme. The helix in illustration b is shown as a part of this structure. A caption pointing to the heading reads, The secondary structure folds further into a tertiary structure.

Part d on the extreme right, with the heading quaternary structure shows four structures of illustration c joined together. A caption pointing to the heading reads, Two or more polypeptide chains may associate to create a quaternary structure.

CONCEPTS

The products of many genes are proteins whose functions produce the traits specified by these genes. Proteins are polymers consisting of amino acids linked by peptide bonds. The amino acid sequence of a protein is its primary structure. This structure folds to create the secondary and tertiary structures; two or more polypeptide chains may associate to create a quaternary structure.

✔ CONCEPT CHECK 2

What primarily determines the secondary and tertiary structures of a protein?

15.2 The Genetic Code Determines How the Nucleotide Sequence Specifies the Amino Acid Sequence of a Protein

In 1953, James Watson, Francis Crick, Rosalind Franklin, and Maurice Wilkins solved the structure of DNA and identified its base sequence as the carrier of genetic information (see Chapter 10). However, the way in which the base sequence of DNA specifies the amino acid sequences of proteins (the genetic code) remained elusive for another 10 years.

One of the first questions about the genetic code to be addressed was how many nucleotides are necessary to specify a single amino acid. The set of nucleotides that encode a single amino acid—the basic unit of the genetic code—is called a *codon* (see Chapter 14). Many early investigators recognized that codons must contain a minimum of three nucleotides. Each nucleotide position in mRNA can be occupied by one of four bases: A, G, C, or U. If a codon consisted of a single nucleotide, only four different codons (A, G, C, and U) would be possible, which is not enough to encode the 20 different amino acids commonly found in proteins. If codons were made up of two nucleotides each (GU, AC, etc.), there would be $4 \times 4 = 16$ possible codons—still not enough to encode all 20 amino acids. With three nucleotides per codon, there are $4 \times 4 \times 4 = 64$ possible codons,

which is more than enough to specify 20 different amino acids. Therefore, a *triplet code* requiring three nucleotides per codon would be the most efficient way to encode all 20 amino acids. Using mutations in bacteriophages, Francis Crick and his colleagues confirmed in 1961 that the genetic code is indeed a triplet code. ›

TRY PROBLEMS 20 AND 21

THINK-PAIR-SHARE Question 4

CONCEPTS

The genetic code is a triplet code, in which three nucleotides encode each amino acid in a protein.

✔ CONCEPT CHECK 3

A codon is

a. one of three nucleotides that encode an amino acid.
b. three nucleotides that encode an amino acid.
c. three amino acids that encode a nucleotide.
d. one of four bases in DNA.

Breaking the Genetic Code

Once it had been firmly established that the genetic code consists of codons that are three nucleotides in length, the next step was to determine which groups of three nucleotides specify which amino

acids. Logically, the easiest way to break the code would have been to determine the base sequence of a piece of RNA, add it to a test tube containing all the components necessary for translation, and allow it to direct the synthesis of a protein. The amino acid sequence of the newly synthesized protein could then be determined, and its sequence could be compared with that of the RNA. Unfortunately, there was no way at that time to determine the nucleotide sequence of a piece of RNA, so indirect methods were necessary to break the code.

THE USE OF HOMOPOLYMERS

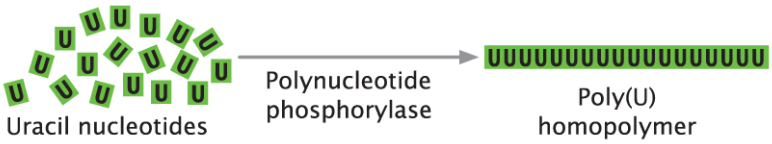
The first clues to the genetic code came in 1961, from the work of Marshall Nirenberg and Johann Heinrich Matthaei. These investigators created synthetic RNAs by using an enzyme called polynucleotide phosphorylase. Unlike RNA polymerase, polynucleotide phosphorylase does not require a template; it randomly links together any RNA nucleotides that happen to be available. The first synthetic mRNAs used by Nirenberg and Matthaei were homopolymers, RNA molecules consisting of a single type of nucleotide. For example, by adding polynucleotide phosphorylase to a solution of uracil nucleotides, they generated RNA molecules that consisted entirely of uracil nucleotides and thus contained only UUU codons (**Figure 15.8**). These poly(U) RNAs were then added to 20 test tubes, each containing the components necessary for translation and all 20 amino acids. A different amino acid was radioactively labeled in each of the 20 tubes. Radioactive protein

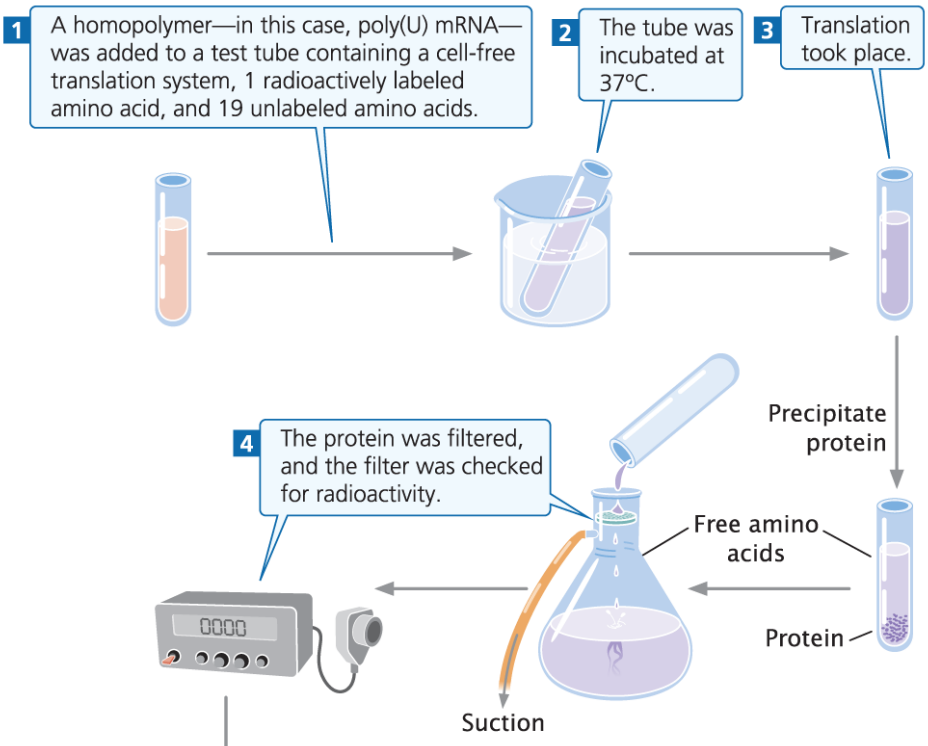
appeared in only one of the tubes—the one containing labeled phenylalanine (see Figure 15.8). This result showed that the codon UUU specifies the amino acid phenylalanine. The results of similar experiments using poly(C) and poly(A) RNA demonstrated that CCC encodes proline and AAA encodes lysine; for technical reasons, the results from poly(G) were uninterpretable.

Experiment

Question: What amino acids are specified by codons composed of only one type of base?

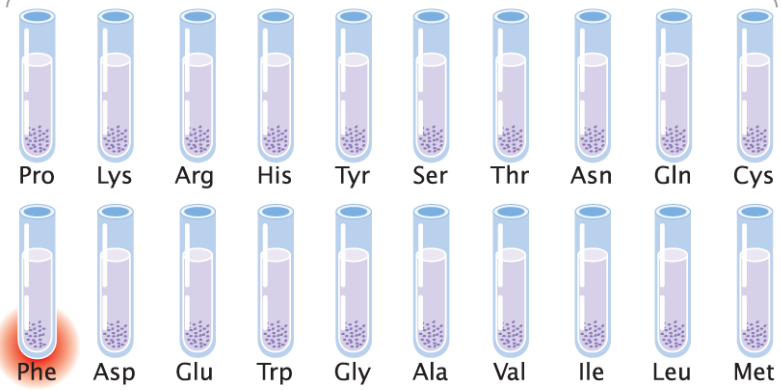
Methods



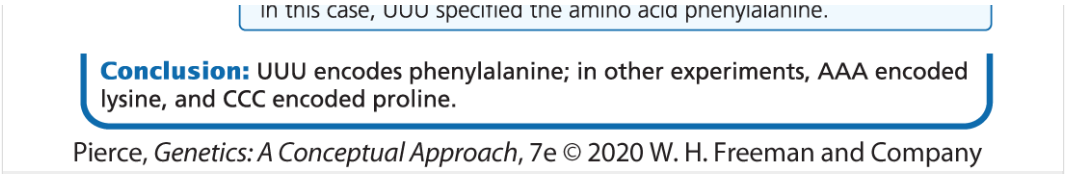


5 The procedure was repeated in 20 tubes, with each tube containing a different labeled amino acid.

Results



6 The tube in which the protein was radioactively labeled contained newly synthesized protein, with the amino acid specified by the homopolymer.



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15.8 Nirenberg and Matthaei developed a method for identifying the amino acid specified by an RNA homopolymer.

Description

The figure begins with a question that reads, What amino acids are specified by codons composed of only one type of base? Methods below show 18 randomly arranged Uracil nucleotides. These nucleotides align in a straight line forming poly (U) homopolymer in the presence of polynucleotide phosphorylase. Below this, a test tube is shown with liquid inside. The first caption reads, A homopolymer, in this case, poly (U) m R N A, was added to a test tube containing a cell-free translation system, 1 radioactively labeled amino acid, and 19 unlabeled amino acids. The test tube is then placed slanting in a beaker with half-filled liquid. The second caption reads, The tube was incubated at 37 degrees Celsius. The illustration then shows test tube with another liquid. A caption pointing towards the test tube reads, Translation took place. In the next step below, protein precipitation takes place and free amino acids are present at the top of the test tube in supernatant, while protein is precipitated at the bottom in pellet. Next, the test tube solution is poured in to a conical flask that has a suction outlet at its neck and is pointing towards filtration equipment. The fourth caption reads, The protein was filtered, and the filter was checked for radioactivity. The results section below shows 20 test tubes having 20 different amino acids. The fifth caption reads, The procedure was repeated in 20 tubes, with each tube containing a different labeled amino acid. The names of 20 amino acids isolated are as follows: Proline, Lysine, Arginine, Histidine, Tyrosine, Serine, Threonine, Asparagine, Glutamine, Cysteine, Phenylalanine, Aspartic acid, Glutamic acid, Tryptophan, Glycine, Alanine, Valline, Isoluecine, Leucine, and Methionine. A sixth caption pointing towards phenylalanine reads, The tube in which the protein was radioactively labeled contained newly synthesized protein with the amino acid specified by the homopolymer. In this case, U U U specified the amino acid phenylalanine. A caption below reads, conclusion: U U U encodes phenylalanine; in other experiments, A A A encoded lysine, and C C C encoded proline.

THE USE OF RANDOM COPOLYMERS

To gain information about additional codons, Nirenberg and his colleagues created synthetic RNAs containing two or three different bases. Because polynucleotide phosphorylase incorporates nucleotides randomly, these RNAs contain random mixtures of the bases and are thus called random copolymers. For example, when adenine and cytosine nucleotides were mixed with polynucleotide phosphorylase, the RNA molecules produced had eight different codons: AAA, AAC, ACC, ACA, CAA, CCA, CAC, and CCC. These poly(AC) RNAs produced proteins containing six different amino acids: asparagine, glutamine, histidine, lysine, proline, and threonine.

The proportions of the different amino acids in the proteins produced depended on the ratio of the two nucleotides used in creating the random copolymers, and the theoretical probability of finding a particular codon could be calculated from the ratios of the bases. If a 4 : 1 ratio of C to A were used in making the RNA, then the probability of C being in any given position in a codon would be $^4/_5$ and the probability of A being in it would be $^1/_5$. With random incorporation of bases, the probability of any one of the codons with two Cs and one A (CCA, CAC, or ACC) would be $^4/_5 \times {}^4/_5 \times {}^1/_5 = {}^{16}/_{125} = 0.13$, or 13%, and the probability of any codon with two As and one C (AAC, ACA, or CAA) would be $^1/_5 \times {}^1/_5 \times {}^4/_5 = {}^4/_{125} = 0.032$, or about 3%. Therefore, an amino acid

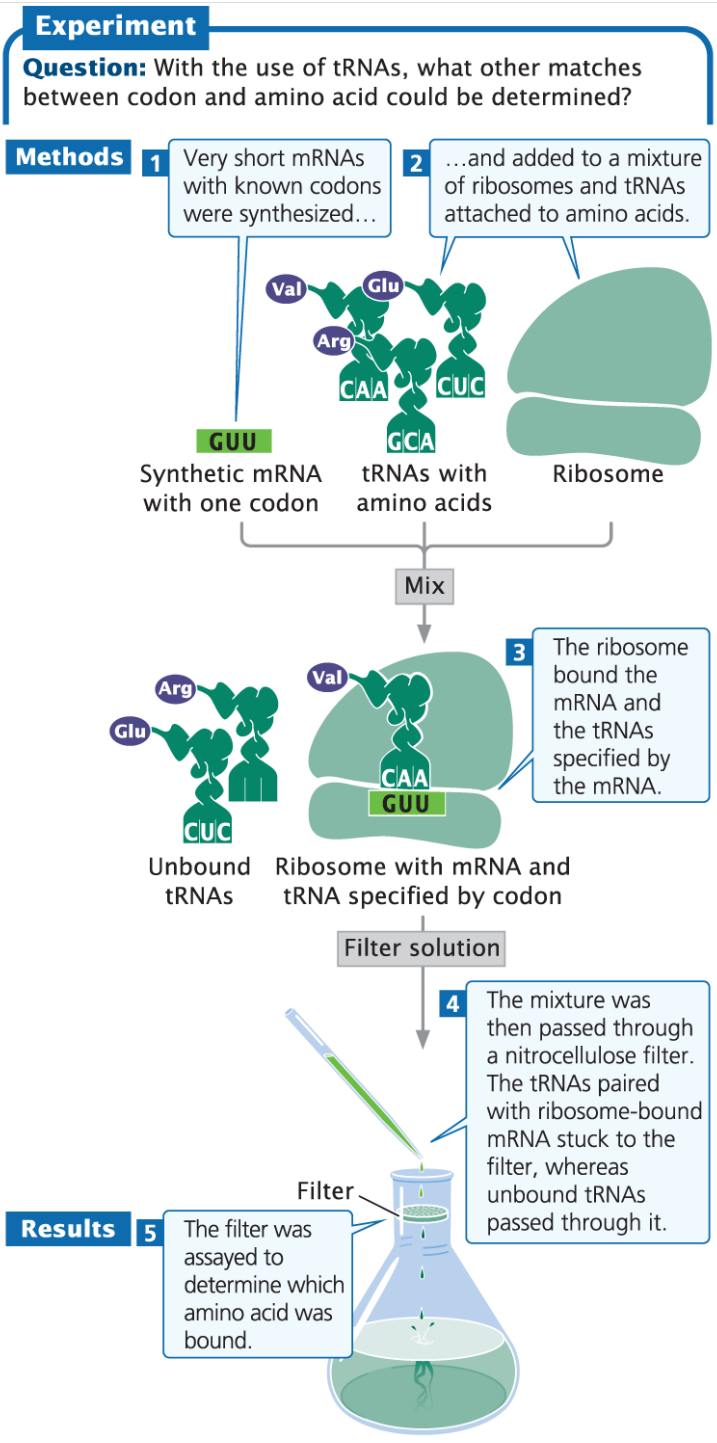
encoded by two Cs and one A should be more common than an amino acid encoded by two As and one C.

By comparing the percentages of amino acids in proteins produced by random copolymers with the theoretical frequencies expected for the codons, Nirenberg and his colleagues could derive information about the base *composition* of the codons. These experiments revealed nothing, however, about the codon base *sequence*; histidine was clearly encoded by a codon with two Cs and one A, but whether that codon was ACC, CAC, or CCA was unknown. There were other problems with this method: the theoretical calculations depended on the random incorporation of bases, which did not always occur; furthermore, because the genetic code is redundant, sometimes several different codons specify the same amino acid.

THE USE OF RIBOSOME-BOUND tRNAs

To overcome the limitations of random copolymers, Nirenberg and Philip Leder developed another technique in 1964 that used ribosome-bound tRNAs. They found that a very short sequence of mRNA—even one consisting of a single codon—would bind to a ribosome. The codon on the short mRNA would then base pair with the matching anticodon on a tRNA that carried the amino acid specified by the codon (Figure 15.9). When short mRNAs that were bound to ribosomes were mixed with tRNAs and amino acids, and this mixture was passed through a nitrocellulose filter, the tRNAs

that were paired with the ribosome-bound mRNA stuck to the filter, whereas unbound tRNAs passed through it. The advantage of this system was that it could be used with very short synthetic mRNA molecules that could be synthesized with a known sequence. Nirenberg and Leder synthesized more than 50 short mRNAs with known codons and added them individually to a mixture of ribosomes and tRNAs with amino acids. They then isolated the tRNAs that were bound to the mRNAs and ribosomes and determined which amino acids were present on the bound tRNAs. For example, synthetic mRNA with the codon GUU retained a tRNA to which valine was attached, whereas mRNAs with the codons UGU and UUG did not. Using this method, Nirenberg and his colleagues were able to determine the amino acids encoded by more than 50 codons.



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15.9 Nirenberg and Leder used ribosome-bound tRNAs to provide additional information about the genetic code.

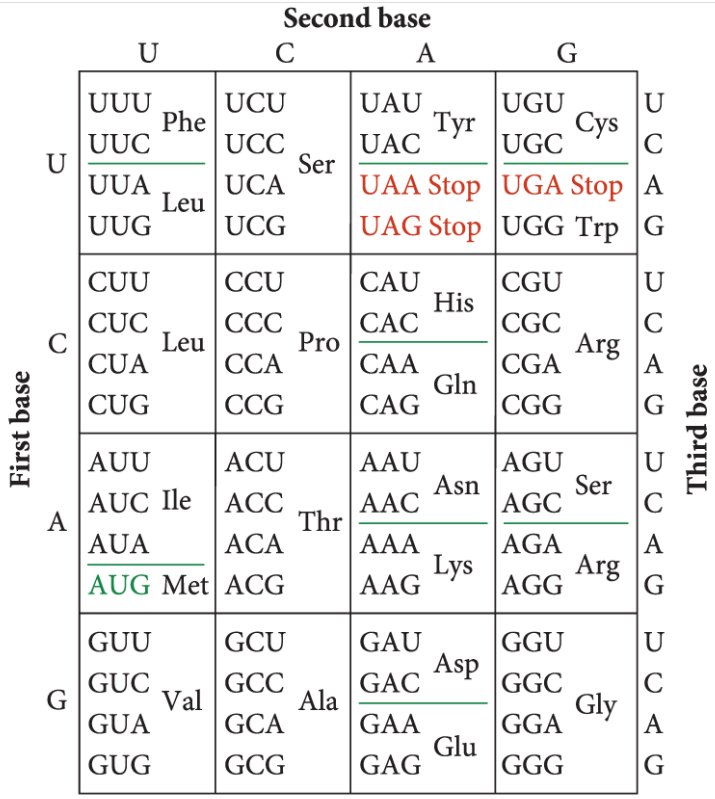
Description

The figure starts with a question that reads, With the use of t R N A s, what other matches between codon and amino acid could be determined? The methods below show G U U, a synthetic m R N A with one codon on the left. A caption reads, Very short m R N A's with known codons were synthesized and added to a mixture of ribosomes and t R N As attached to amino acids. At the center, three bound t R N A's attached with their respective amino acids are as follows: C A A, Valine; G C A, Arginine; and C U C, Glutamic acid. On the right, part shows the small and large units of a ribosome. An arrow pointing downward shows mixing of m R N A with t R N A's and ribosome. Next, unbounded t R N A's with their respective amino acids are as follows: G C A, Arginine; and C U C, Glutamic acid. Beside this is ribosome with m R N A and t R N A specified by codon. It shows synthetic m R N A, G U U on the small subunit of ribosome and t R N A, C A A attached with Valine above the m R N A on the large subunit of ribosome. A caption reads, The ribosome bound the m R N A and the t R N A's specifi¬ed by the m R N A. Next, Ribosome with m R N A and t R N A specified by codon undergo formation of filter solution below. Results section below shows drops falling from a pipette in to a conical flask with a filter at its neck. Two captions read as follows, The mixture was then passed through a nitrocellulose-filter. The t R N A's paired with ribosome-bound m R N A stuck to the fi¬lter, whereas unbound t R N A's passed through it and The filter was assayed to determine which amino acid was bound. A caption below reads, Conclusion: When an m R N A with G U U was added, the t R N A s on the filter were bound to valine; therefore, the codon G U U specifies valine. Many other codons were determined by using this method.

Other experiments provided additional information about the genetic code, and it was fully deciphered by 1968. Let's examine some of the features of the code, which is so important to modern biology that Francis Crick compared its place to that of the periodic table of the elements in chemistry.

The Degeneracy of the Code

One amino acid is encoded by three consecutive nucleotides in mRNA, and each nucleotide can have one of four possible bases (A, G, C, or U), so there are $4^3 = 64$ possible codons (**Figure 15.10**). Three of these codons are stop codons, which specify the end of translation, as we'll see shortly. Thus, 61 codons, called **sense codons**, encode amino acids. Because there are 61 sense codons and only 20 different amino acids commonly found in proteins, the code contains more information than is needed to specify the amino acids and thus is said to be **degenerate**. This expression does not mean that the genetic code is depraved; *degenerate* is a term that Francis Crick borrowed from quantum physics, where it describes multiple physical states that have equivalent meaning. The degeneracy of the genetic code means that the code is redundant: amino acids may be specified by more than one codon. Only tryptophan and methionine are encoded by a single codon (see Figure 15.10). Other amino acids are specified by two or more codons, and some, such as leucine, are specified by six different codons. Codons that specify the same amino acid are said to be **synonymous codons**, just as synonymous words are different words that have the same meaning.



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15.10 The genetic code consists of 64 codons. The amino acids specified by each codon are given in their three-letter abbreviation. The codons are written 5′→3′, as they appear in the mRNA. AUG is an initiation (start) codon as well as the codon for methionine; UAA, UAG, and UGA are termination (stop) codons.

Description

The column headers and left row headers for each of the four columns and rows are as follows U, C, A, and G. The right row headers for each row are U C A G, U C A G, U C A G, and U C A G. The combined left row heading is first base, combined column heading is second base, and combined right row heading is third base. The entries are depicted as follows:

Row 1 column 1: U U U and U U C (codes for phenylalanine); U U A and U U G (codes for leucine)

Row 1 column 2: U C U, U C C, U C A, U C G (codes for serine)

Row 1 column 3: U A U and U A C (codes for tyrosine); U A A and U A G (highlighted in red and represent stop codon)

Row 1 column 4: U G U and U G C (codes for cysteine); U G A (highlighted in red and represents stop codon); U G G (codes for tryptophan)

Row 2 column 1: C U U, C U C, C U A , C U G (codes for leucine)

Row 2 column 2: C C U, C C C, C C A, C C G (codes for proline)

Row 2 column 3: C A U and C A C (codes for histidine); C A A and C A G (codes for glutamine)

Row 2 column 4: C G U, C G C, C G A, C G G (codes for arginine)

Row 3 column 1: A U U, A U C, and A U A (codes for isoleusine); A U G (codes for methionine)

Row 3 column 2: A C U, A C C, A C A, A C G (codes for threonine)

Row 3 column 3: A A U and A A C (codes for asparagines); A A A and A A G (codes for lysine)

Row 3 column 4: A G U and A G C (codes for serine), A G A and A G G (codes for arginine)

Row 4 column 1: G U U, G U C, G U A, G U G (codes for valine)

Row 4 column 2: G C U, G C C, G C A, G C G (codes for alanine)

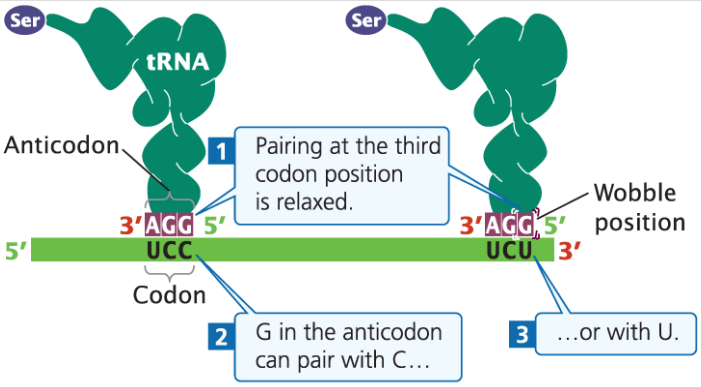
Row 4 column 3: G A U and G A C (codes for aspartic acid); G A A and G A G (codes for glutamic acid)

Row 4 column 4: G G U, G G C, G G A, G G G (codes for glycine).

As we learned in Chapter 14, tRNAs serve as adapter molecules that bind particular amino acids and deliver them to a ribosome, where the amino acids are then assembled into polypeptide chains. Each type of tRNA attaches to a single type of amino acid. The cells of

most organisms possess about 30 to 50 different tRNAs, yet there are only 20 different amino acids commonly found in proteins. Thus, some amino acids are carried by more than one tRNA. Different tRNAs that accept the same amino acid but have different anticodons are called **isoaccepting tRNAs**.

Even though some amino acids can pair with multiple (isoaccepting) tRNAs, there are still more codons than anticodons. One anticodon can pair with different codons through flexibility in base pairing at the third position of the codon. Examination of Figure 15.10 reveals that many synonymous codons differ only in the third position. For example, serine is encoded by the codons UCU, UCC, UCA, and UCG, all of which begin with UC. When the codon of the mRNA and the anticodon of the tRNA join (**Figure 15.11**), the first (5′) base of the codon forms hydrogen bonds with the third (3′) base of the anticodon, strictly according to the Watson-and-Crick base-pairing rules: A with U; C with G. Next, the middle bases of codon and anticodon pair, also strictly following the Watson-and-Crick rules. After these pairs have bonded, the third bases pair weakly, and there may be flexibility, or **wobble**, in their pairing.



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15.11 Wobble may exist in the pairing of a codon and an anticodon. The mRNA and tRNA pair in an antiparallel fashion. Pairing at the first and second codon positions is in accord with the Watson-and-Crick rules (A with U, G with C); however, pairing rules are relaxed at the third position of the codon, and G on the anticodon can pair with either U or C on the codon in this example.

Description
The diagram shows a 5 prime to 3 prime end m R N A strand. A t R N A attached with amino acid serine and anticodon sequence A G G from 3 prime to 5 prime end is sitting on the codon U C C of m R N A strand towards its 5 prime end. First caption reads, Pairing at the third codon position is relaxed. Second caption reads, G in the anticodon can pair with C or with U. Next, a t R N A attached with amino acid serine and anticodon sequence A G G from 3 prime to 5 prime end is sitting on the codon U C U of the m R N A strand towards its 3 prime end. Third position of codon and anticodon pairing at the 3 prime end shows wobble position where G is pairing with U.

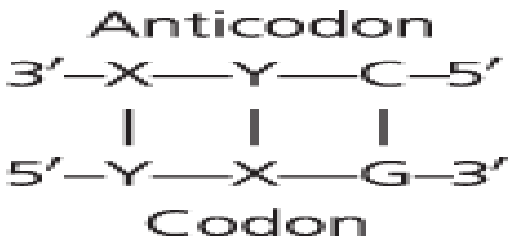
In 1966, Francis Crick developed the wobble hypothesis, which proposed that some nonstandard pairings of bases could take place at the third position of a codon. For example, a G in the anticodon may pair with either a C or a U in the third position of the codon (Table 15.2). The important thing to remember about wobble is that it allows some tRNAs to pair with more than one mRNA codon; thus,

from 30 to 50 tRNAs can pair with 61 sense codons. Some codons are synonymous through wobble. ❯ **TRY** PROBLEM 26

THINK-PAIR-SHARE Question 5

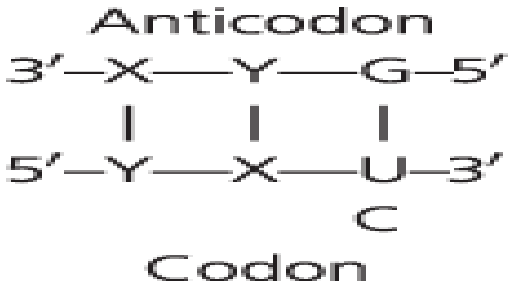
TABLE 15.2 The wobble rules, indicating which bases in the third position (3′ end) of an mRNA codon can pair with which bases at the first position (5′ end) of a tRNA anticodon

First Position of Anticodon	Third Position of Codon	Pairing
C	G	



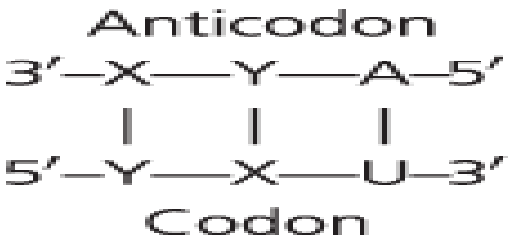
Description
The anticodon sequence runs from 3 prime to 5 prime and has the following sequence: X, Y, and C. The codon sequence below runs from 5 prime to 3 prime and has the following corresponding sequence: Y, X, and G.

G	U or C



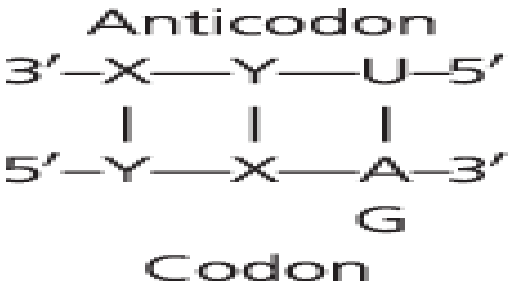
Description
The anticodon sequence runs from 3 prime to 5 prime and has the following sequence: X, Y, and G. The codon sequence below runs from 5 prime to 3 prime and has the following corresponding sequence: Y, X, and U. A nucleotide C is present below U nucleotide of codon sequence.

A	U

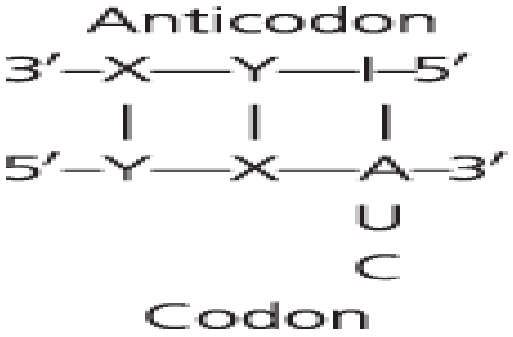


Description
The anticodon sequence runs from 3 prime to 5 prime and has the following sequences: X, Y, and A. The codon sequence below runs from 5 prime to 3 prime and has the

following corresponding sequence: Y, X, and U.

U	A or G	

Description
The anticodon sequence runs from 3 prime to 5 prime and has the following sequence: X, Y, and U. The codon sequence below runs from 5 prime to 3 prime and has the following corresponding sequence: Y, X, and A. A nucleotide G is present below A nucleotide of codon sequence.

I (inosine)*	A, U, or C	

Description
The anticodon sequence runs from 3 prime to 5 prime and has the following sequences: X, Y, and I. The codon sequence below runs from 5 prime to 3 prime and has the following corresponding sequence: Y, X, and A. Two nucleotides U and C are present below A nucleotide of the codon sequence.

---

*Inosine is one of the modified bases found in tRNAs.

CONCEPTS

The genetic code includes 61 sense codons that specify the 20 common amino acids. The code is degenerate, meaning that some amino acids are encoded by more than one codon. Isoaccepting tRNAs are tRNAs with different anticodons that specify the same amino acid. Wobble at the third position of the codon allows different codons to specify the same amino acid.

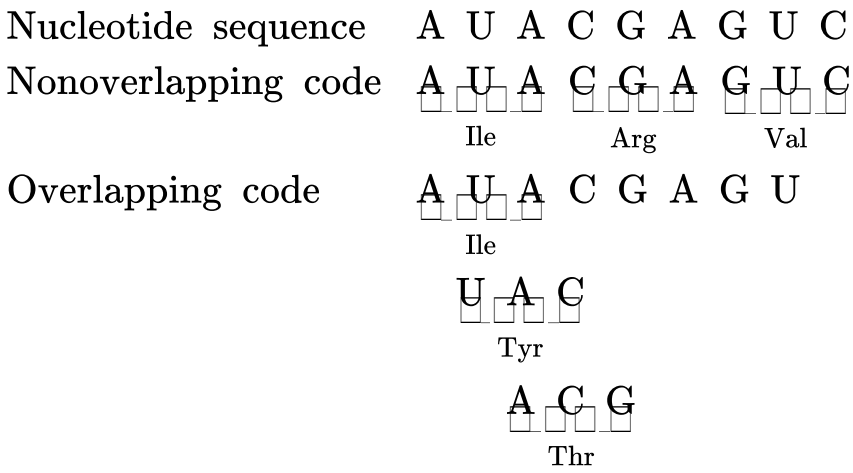
✔ CONCEPT CHECK 4

Through wobble, a single _____ can pair with more than one _____.

a. codon, anticodon
b. group of three nucleotides in DNA, codon in mRNA
c. tRNA, amino acid
d. anticodon, codon

The Reading Frame and Initiation Codons

Findings from early studies of the genetic code indicated that the code is generally **nonoverlapping**. An overlapping code would be one in which a single nucleotide might be included in more than one codon, as follows:



Usually, however, each nucleotide is part of a single codon. A few overlapping genes are found in viruses, but codons within the same gene do not overlap, and the genetic code is generally considered to be nonoverlapping.

For any sequence of nucleotides, there are three potential sets of codons—three ways in which the sequence can be read in groups of three. Each different way of reading the sequence is called a **reading frame**, and any sequence of nucleotides has three potential reading frames. The three reading frames have completely different sets of

codons and therefore specify proteins with entirely different amino acid sequences. Thus, it is essential for the translation machinery to use the correct reading frame. How is the correct reading frame established? The reading frame is set by the **initiation codon** (or **start codon**), which is the first codon of the mRNA to specify an amino acid. After the initiation codon, the other codons are read as successive groups of three nucleotides. No bases are skipped between the codons, so there are no punctuation marks to separate the codons.

The initiation codon is most often AUG, although GUG, UUG, and other codons are also sometimes used. The initiation codon is not just a sequence that marks the beginning of translation; it also specifies an amino acid. In bacterial cells, the first AUG encodes a modified type of methionine, *N*-formylmethionine; thus, all proteins in bacteria initially begin with this amino acid, but its formyl group (or, in some cases, the entire amino acid) may be removed after the protein has been synthesized. When the codon AUG is at an internal position in a gene, it encodes unformylated methionine. In archaeal and eukaryotic cells, AUG specifies unformylated methionine, both at the initiation position and at internal positions. In both bacteria and eukaryotes, there are different tRNAs for the initiator methionine (designated $tRNA_i^{fMet}$ in bacteria and $tRNA_i^{Met}$ in eukaryotes) and internal methionine (designated $tRNA^{Met}$).

Termination Codons

Three codons—UAA, UAG, and UGA—do not encode amino acids. These codons, which signal the end of translation in both bacterial and eukaryotic cells, are called **stop codons**, **termination codons**, or **nonsense codons**. No tRNAs have anticodons that pair with termination codons.

THINK-PAIR-SHARE Question 6

The Universality of the Code

For many years, the genetic code was assumed to be **universal**, meaning that each codon specifies the same amino acid in all organisms. We now know that the genetic code is mostly, but not completely, universal; some exceptions have been found. Most of these exceptions are termination codons, but there are a few cases in which one sense codon substitutes for another. Many of these exceptions are found in mitochondrial genes; some nonuniversal codons have also been detected in the nuclear genes of protozoans and in bacterial DNA (Table 15.3). One study of bacteria and bacteriophages isolated from 1776 environmental samples found nonuniversal codons in a substantial fraction, suggesting that nonuniversal codons may be more common than previously thought. ❯ **TRY PROBLEM 22**

THINK-PAIR-SHARE Question 7

TABLE 15.3 Some exceptions to the universal genetic code

Genome	Codon	Universal Code	Altered Code
Bacterial DNA			
Mycoplasma capricolum	UGA	Stop	Trp
Mitochondrial DNA			
Human	UGA	Stop	Trp
Human	AUA	Ile	Met
Human	AGA, AGG	Arg	Stop
Yeast	UGA	Stop	Trp
Trypanosomes	UGA	Stop	Trp
Plants	CGG	Arg	Trp
Nuclear DNA			
Tetrahymena	UAA	Stop	Gln

Paramecium	UAG	Stop	Gln

CONCEPTS

Each sequence of nucleotides possesses three potential reading frames. The correct reading frame is set by the initiation codon. The end of a protein-coding sequence is marked by a termination codon. With a few exceptions, all organisms use the same genetic code.

✔ CONCEPT CHECK 5

Do the initiation and termination codons specify amino acids? If so, which ones?

CONNECTING CONCEPTS

Characteristics of the Genetic Code

We have now considered a number of characteristics of the genetic code. Let's take a moment to review these characteristics.

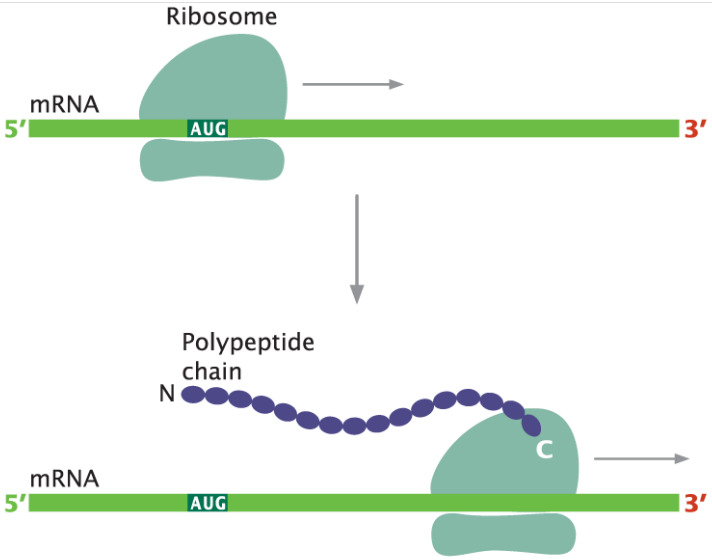
1. The genetic code consists of a sequence of nucleotides in DNA or RNA. There are four letters in the code, corresponding to the four bases—A, G, C, and U (T in DNA).
2. The genetic code is a triplet code. Each amino acid is encoded by a sequence of three consecutive nucleotides, called a codon.
3. The genetic code is degenerate: of 64 codons, 61 codons encode only 20 amino acids in proteins (3 codons are termination codons). Some codons are synonymous, specifying the same amino acid.
4. Isoaccepting tRNAs are tRNAs with different anticodons that accept the same amino acid. Wobble allows the anticodon on one type of tRNA to pair with more than one type of codon on mRNA.
5. The genetic code is generally nonoverlapping; each nucleotide in an mRNA sequence belongs to a single reading frame.
6. The reading frame is set by an initiation codon, which is usually AUG.
7. When a reading frame has been set, codons are read as successive groups of three nucleotides.

8. Any one of three termination codons (UAA, UAG, or UGA) can signal the end of translation; no amino acids are encoded by the termination codons.
9. The genetic code is almost universal.

15.3 Amino Acids Are Assembled into a Protein Through Translation

Now that we are familiar with the genetic code, we can begin to study how amino acids are assembled into proteins. Because more is known about translation in bacteria than in eukaryotes, we will focus primarily on bacterial translation. In most respects, eukaryotic translation is similar, although some significant differences will be noted.

Remember that only mRNAs are translated into proteins. Translation takes place on ribosomes; indeed, ribosomes can be thought of as moving protein-synthesizing machines. Through a variety of techniques, a detailed view of the structure of the ribosome has been produced in recent years, which has greatly improved our understanding of translation. A ribosome attaches near the $5'$ end of an mRNA strand and moves toward the $3'$ end, translating the codons as it goes (**Figure 15.12**). Synthesis begins at the amino end of the protein, and the protein is elongated by the addition of new amino acids to the carboxyl end. Protein synthesis includes a series of RNA–RNA interactions: interactions between the mRNA and the rRNA that holds the mRNA in the ribosome, between the codon on the mRNA and the anticodon on the tRNA, and between the tRNA and the rRNAs of the ribosome.



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15.12 The translation of an mRNA molecule takes place on a ribosome. The letter N represents the amino end of the protein; C represents the carboxyl end.

Description

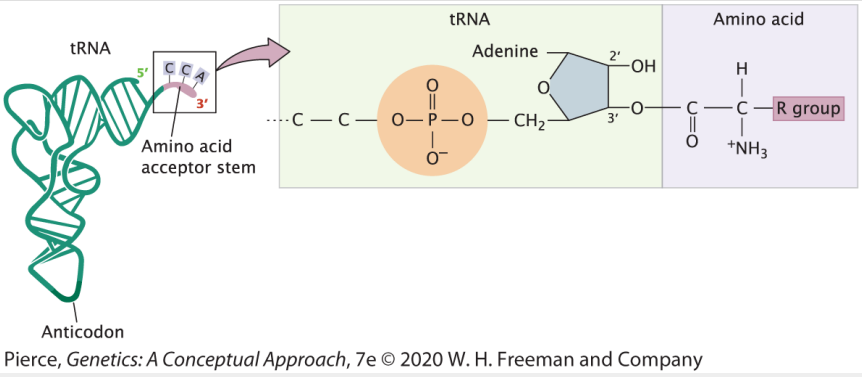
The diagram shows a single stranded m R N A that runs from 5 prime to 3 prime. A ribosome is sitting on the A U G site of an m R N A strand near the 5 prime end of the strand with its larger subunit above and smaller subunit below the strand. An arrow from the ribosome is pointing forward from 5 prime to 3 prime. It leads to next step below where the movement of the ribosome to the 3 prime end of the m R N A strand is shown. A growing polypeptide chain is now attached to the large subunit of the ribosome. The end of the polypeptide that is attached to the ribosome is labeled C, while the free end is labeled N. The A U G site is on the same location on strand as before.

Protein synthesis can be conveniently divided into four stages: (1) tRNA charging, in which tRNAs bind to amino acids; (2) initiation, in which the components necessary for translation are assembled at the ribosome; (3) elongation, in which amino acids are joined, one at a time, to the growing polypeptide chain; and (4) termination, in

which protein synthesis halts at the termination codon and the translation components are released from the ribosome.

The Binding of Amino Acids to Transfer RNAs

The first stage of translation is the binding of tRNA molecules to their appropriate amino acids. As we have seen, each tRNA is specific for a particular amino acid. All tRNAs have the sequence CCA at the 3′ end, and the carboxyl group (COO^-) of the amino acid is attached to the adenine nucleotide at the 3′ end of the tRNA (Figure 15.13). If each tRNA is specific for a particular amino acid but all amino acids are attached to the same nucleotide (A) at the 3′ end of a tRNA, how does a tRNA link up with its appropriate amino acid?



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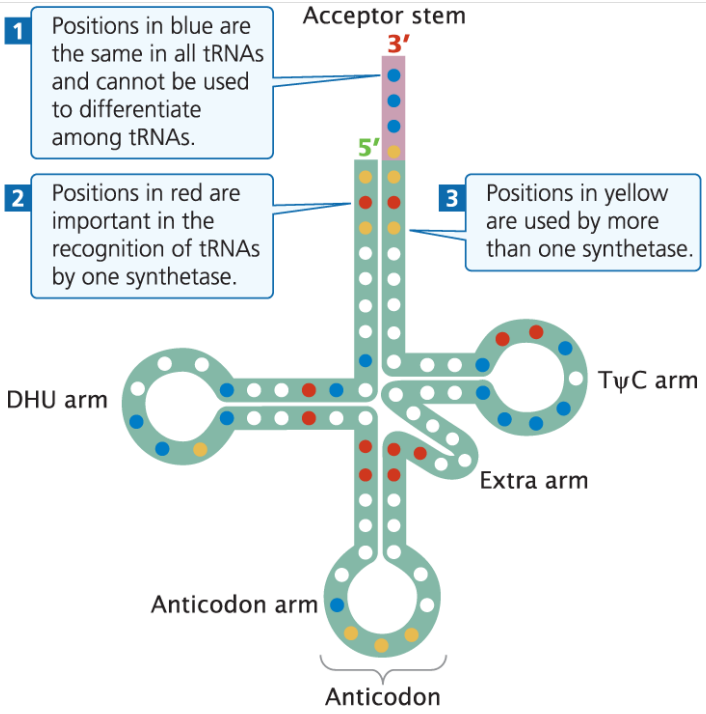
15.13 An amino acid attaches to the 3′ end of a tRNA. The carboxyl group (COO^-) of the amino acid attaches to the hydroxyl group (OH) of the 2′- or 3′-carbon atom of the final nucleotide at the 3′ end of the tRNA, in which the base is always adenine.

Description
The first part shows a ribbon model of a t R N A strand, on the left, with its ends labeled as 5 prime and 3 prime. An anticodon region is marked at the bottom of the model. The 3 prime end of the t R N A strand has an amino acid acceptor stem with nucleotides C, C, A.

The second part of the illustration, on the right, represents a magnified view of bonding.

At the center of the structure, a pentagon is present. The carbon at 1 prime position is bonded to adenine. The carbon at 2 prime position is bonded to hydroxyl group (O H) on the right. The carbon at 3 prime position is bonded to the carboxyl group on the right which is further bonded to carbon on the right that is bonded to hydrogen atom and an ammonia cation at the top and bottom respectively and an R group on the right. The carbon at 4 prime position is bonded to methylene group on the left that is further bonded to phosphate group on the left. Phosphate group is bonded to carbon on the left that is further bonded to carbon on the left. The carbon at 5 prime position is bonded to oxygen atom. The groups including the carboxyl group, and the groups it is is further bonded to is labeled amino acid. All the remaining part of the structure is labeled t R N A.

The key to specificity between an amino acid and its tRNA is a set of enzymes called **aminoacyl-tRNA synthetases**. A cell has 20 different aminoacyl-tRNA synthetases, one for each of the 20 amino acids. Each synthetase recognizes a particular amino acid as well as all the tRNAs that accept that amino acid. Its recognition of the appropriate amino acid is based on the different sizes, charges, and R groups of the amino acids. Its recognition of the appropriate tRNAs depends on the nucleotide sequences of the tRNAs. Researchers have identified which nucleotides are important in recognition by synthetases by altering different nucleotides in a particular tRNA and determining whether the altered tRNA is still recognized by its synthetase (**Figure 15.14**).



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15.14 Certain positions on tRNA molecules are recognized by the appropriate aminoacyl-tRNA synthetase.

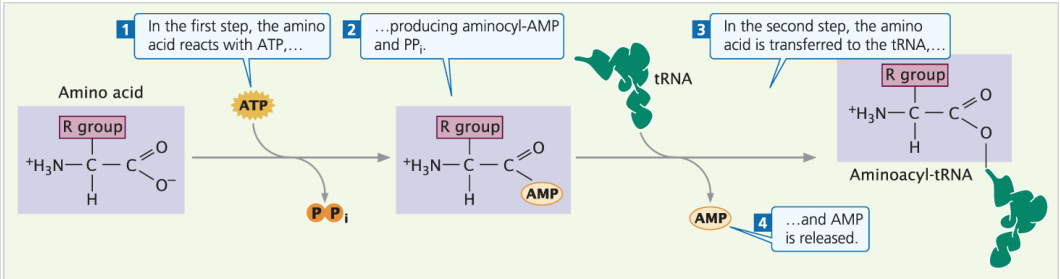
Description

The diagram shows the cloverleaf structure of a t R N A with the D H U arm on the left and the T psi C arm the right. A small extra arm of variable size projects out between the T psi C arm and the anticodon arm at the bottom of the structure, on the right side. Few blue, yellow, red, and white colored spheres are scattered throughout the structure. At the top of the cloverleaf structure, the acceptor stem is labeled at 3 prime end. The bottom part consists of yellow spheres which represent an anticodon and a blue sphere which represents the anticodon arm. A caption pointing to the acceptor stem of the 3 prime end reads Positions in blue are the same in all t R N A's and cannot be used to differentiate among t R N A's. The second caption at 5 prime end of the cloverleaf structure parallel to 3 prime end and below the acceptor stem portion reads, Positions in red are important in the recognition of t R N A's by one synthetase. A caption pointing at the 3 prime end and below acceptor stem portion reads, Positions in yellow are used by more than one synthetase.

The attachment of a tRNA to its appropriate amino acid, termed **tRNA charging**, requires energy, which is supplied by adenosine triphosphate (ATP):

amino acid + tRNA + ATP → aminoacyl-tRNA + AMP + PPi

This reaction takes place in two steps (**Figure 15.15**). To identify the resulting aminoacylated (charged) tRNA, we write the three-letter abbreviation for the amino acid in front of the tRNA; for example, the amino acid alanine (Ala) attaches to its tRNA ($tRNA^{Ala}$), giving rise to its aminoacyl-tRNA ($Ala\text{-}tRNA^{Ala}$).



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15.15 An amino acid becomes attached to the appropriate tRNA in a two-step reaction.

Description

The diagram starts with an amino acid, on the left, having the configuration as follows:

A carbon atom is bonded to R group and H atom on top and at bottom respectively, ammonia cation is on the left and carboxylate anion is on the right. A forward arrow from this amino acid points to another amino acid and represents the addition of A T P and release of P P i. A caption pointing towards A T P reads, In the first step, the amino acid reacts with A T P producing aminocyl-A M P and P P i.

The configuration of the second amino acid, in the center, shows O superscript negative of the carboxylate anion of the first amino acid is replaced by A M P.

A forward arrow from the second amino acid represents the addition of t R N A and the release of A M P and leads to aminoacyl- t R N A on the right.

Another caption reads, In the second step, the amino acid is transferred to the t R N A and A M P is released.

The configuration of aminoacyl-t R N A, on the right, shows A M P in the second amino and is replaced by single bond O attached to a t-R N A.

A caption below reads, conclusion: At the end of t R N A charging, an amino acid is linked to its appropriate t R N A.

Errors in tRNA charging are rare: they occur in only about 1 in 10,000 to 1 in 100,000 reactions. This fidelity is due in part to the presence of editing (proofreading) activity in many of the aminoacyl-tRNA synthetases. Editing activity detects incorrectly paired amino acids and removes them from the tRNAs. Some antifungal chemical agents work by trapping tRNAs in the editing site of the synthetases, preventing their release and thus inhibiting the process of translation in the fungi.

CONCEPTS

Amino acids are attached to their specific tRNAs by aminoacyl-tRNA synthetases in a two-step reaction that requires ATP.

✔ CONCEPT CHECK 6

Amino acids bind to which part of the tRNA?

a. Anticodon
b. DHU arm
c. 3′ end
d. 5′ end

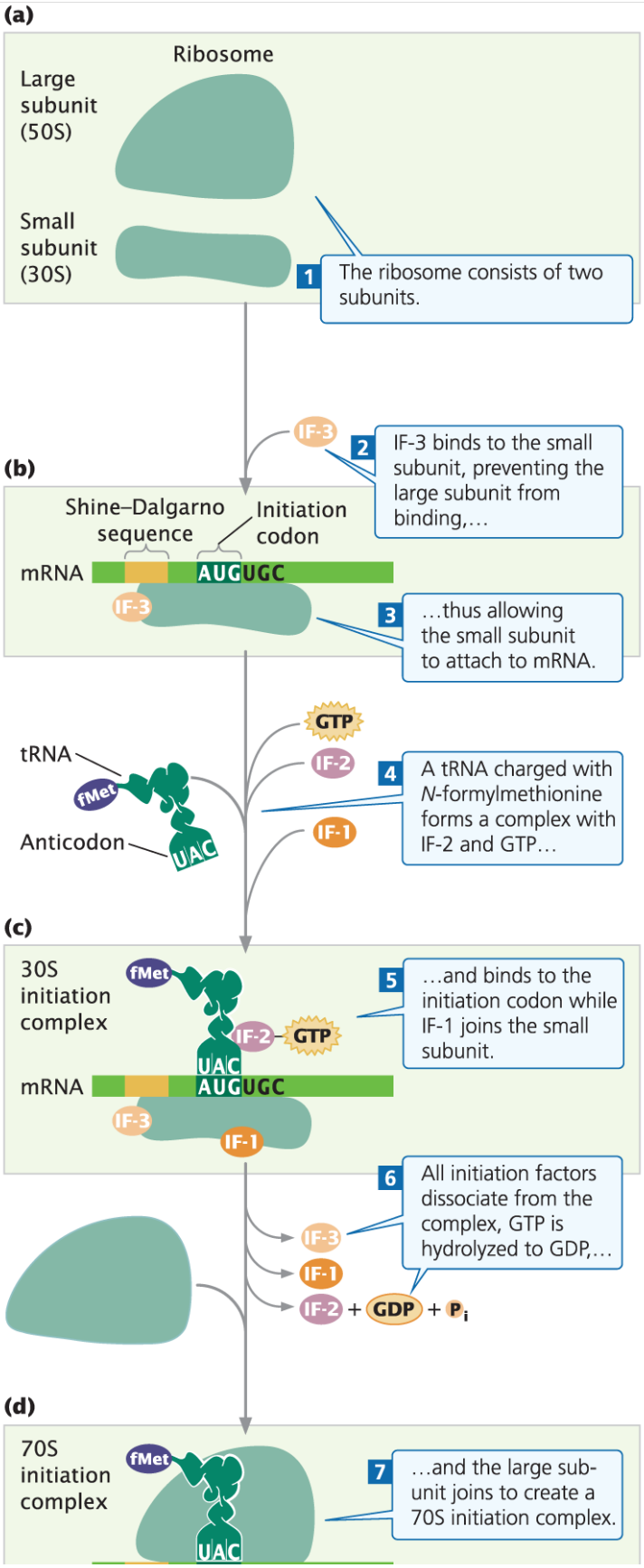
The Initiation of Translation

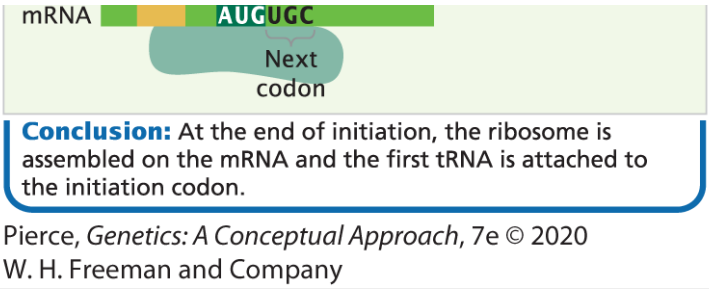
The second stage in the process of protein synthesis is initiation. At this stage, all the components necessary for protein synthesis assemble: (1) mRNA, (2) the small and large subunits of the ribosome, (3) a set of three proteins called initiation factors, (4) initiator tRNA with *N*-formylmethionine attached (fMet-tRNA$_i^{fMet}$), and (5) guanosine triphosphate (GTP). Initiation comprises three major steps. First, mRNA binds to the small subunit of the ribosome. Second, initiator tRNA binds to the mRNA through base pairing between the initiation codon and the anticodon. Third, the large ribosomal subunit joins the initiation complex. Let's look at each of these steps more closely.

INITIATION IN BACTERIA

The functional ribosome of bacteria exists as two subunits, the small 30S subunit and the large 50S subunit (**Figure 15.16a**). An mRNA molecule can bind to the small ribosomal subunit only when the subunits are separate. **Initiation factor 3 (IF-3)** binds to the small

ribosomal subunit and prevents the large subunit from binding during initiation (**Figure 15.16b**). Another factor, **initiation factor 1 (IF-1)**, enhances the disassociation of the large and small ribosomal subunits.


(a)
Ribosome
Large subunit (50S)
Small subunit (30S)
1 The ribosome consists of two subunits.
IF-3
2 IF-3 binds to the small subunit, preventing the large subunit from binding,…
(b)
Shine–Dalgarno sequence
Initiation codon
mRNA
AUGUGC
IF-3
3 …thus allowing the small subunit to attach to mRNA.
GTP
IF-2
4 A tRNA charged with N-formylmethionine forms a complex with IF-2 and GTP…
tRNA
fMet
Anticodon
UAC
IF-1
(c)
30S initiation complex
fMet
IF-2
GTP
UAC
5 …and binds to the initiation codon while IF-1 joins the small subunit.
mRNA
AUGUGC
IF-3
IF-1
6 All initiation factors dissociate from the complex, GTP is hydrolyzed to GDP,…
IF-3
IF-1
IF-2 + GDP + Pi
(d)
70S initiation complex
fMet
UAC
7 …and the large subunit joins to create a 70S initiation complex.



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15.16 The initiation of translation requires several initiation factors and GTP.

Description

Part (a) at the top shows two subunits of ribosome including a large subunit (50s) and a small subunit (30s). A caption reads, The ribosome consists of two subunits.

Part (b) below shows an arrow from part (a) moving downward depicting the addition of I F 3. Another caption reads, I F-3 binds to the small subunit, preventing the larger subunit from binding: thus allowing the small subunit to attach to m R N A.

An m R N A strand is sitting on the small sub-unit of ribosome and, from left to right on the strand, there is a Shine-Dalgarno sequence followed by an initiation codon A U G, then nucleotides U, G, and C. I F 3 is attached to left edge of the small subunit of ribosome. A downward arrow from part (b) leads to part (c) and represents addition of G T P, I F-2, I F-1, and a t R N A with anticodon U A C and an attached amino acid f methionine.

The next caption reads, A t R N A charged with N-formylmethionine forms a complex with I F-2 and G T P and binds to the initiation codon while I F-1 joins the small subunit.

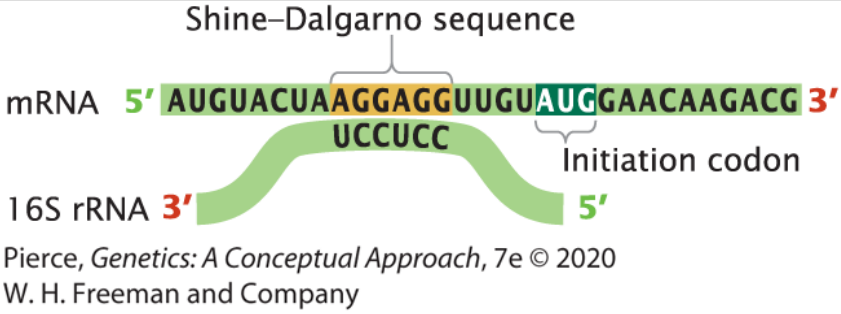
Part (c) below shows an m R N A strand sitting on the small unit of ribosome and consists of an initiation codon A U G at its center followed by nucleotides U, G, and C. A t R N A with anticodon U A C at its bottom and attached to amino acid f methionine on its left arm sitting on the initiation codon and I F-2 is adding on the right side of the t R N A. I F-2 is further attached to G T P on the right. I F 3 and I F 1 are attached at left terminal and central part of the small subunit of ribosome. A text at the top left corner of the illustration reads: 30 S initiation complex.

A downward arrow from part (c) leads to part (d) and represents the removal of I F-3, I F-2, and I F-1 along with G D P and P i, and insertion of large subunit of ribosome. A caption reads: All initiation factors dissociate from the complex, G T P is hydrolyzed to G D P and the large subunit joins to create a 70 S initiation complex.

Part (d) below shows an m R N A strand sitting on the small unit of ribosome and consists of an initiation codon A U G at its center followed by codon U G C. A t R N A with anticodon U A C at its bottom and attached to amino acid along the initiation codon. The large subunit of the ribosome is sitting on the top of the small subunit. Text at the top left corner of the illustration reads: 70 S initiation complex.

A caption below reads, conclusion: At the end of initiation, the ribosome is assembled on the m R N A and the first t R N A is attached to the initiation codon.

Where on the mRNA does the ribosome bind during initiation of translation? Key sequences on the mRNA required for ribosome binding have been identified by techniques designed to allow a ribosome to bind to mRNA, but not to proceed with protein synthesis; the ribosome is thereby stalled at the initiation site where binding occurs. A ribonuclease is added, which degrades all the mRNA except the region covered by the ribosome. The intact mRNA can then be separated from the ribosome and studied. The sequence covered by the ribosome during initiation is 30 to 40 nucleotides long and includes the AUG initiation codon. Within the ribosome-binding site is the Shine–Dalgarno sequence (**Figure 15.17**; see also Chapter 14), a consensus sequence that is complementary to a sequence of nucleotides at the 3′ end of 16S rRNA (part of the small ribosomal subunit). During initiation, the nucleotides in the Shine–Dalgarno sequence pair with their complementary nucleotides in the 16S rRNA, allowing the small ribosomal subunit to attach to the mRNA and positioning the ribosome directly over the initiation codon. These ribosome-binding sequences are within the 5′ untranslated region of the mRNA.



15.17 The Shine–Dalgarno sequence in mRNA is required for the attachment of the small ribosomal subunit. The Shine–Dalgarno consensus sequence base pairs with the 16S rRNA.

Description

The illustration shows an m R N A strand with nucleotides arranged from 5 prime to 3 prime end as: A, U, G, U, A, C, U, A, A, G, G, A, G, G, U, U, G, U, A, U, G, G, A, A, C, A, A, G, A, C, G.

The Shine-Dalgarno consensus sequence from 5 prime to 3 prime is A G G A G G, which is complementary to the U C C U C C sequence of a 16 S r R N A with 3 prime to 5 prime end strand. The initiation codon A U G is present on the right side of Shine-Dalgarno sequence.

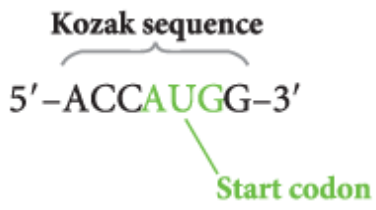
The initiator tRNA, fMet-tRNA$_i^{fMet}$, attaches to the initiation codon (Figure 15.16c). This attachment requires **initiation factor 2 (IF-2)**, which forms a complex with GTP.

At this point, the initiation complex consists of (1) the small ribosomal subunit, (2) the mRNA, (3) the initiator tRNA with its amino acid (fMet-tRNA$_i^{fMet}$), (4) one molecule of GTP, and (5) several initiation factors. These components are collectively known as the **30S initiation complex** (see Figure 15.16c). In the final step of initiation, IF-3 dissociates from the small subunit, allowing the large

ribosomal subunit to join the initiation complex. The molecule of GTP (provided by IF-2) is hydrolyzed to guanosine diphosphate (GDP), and the initiation factors dissociate from the complex (**Figure 15.16d**). When the large subunit has joined the initiation complex, the complex is called the **70S initiation complex**.

INITIATION IN EUKARYOTES

Similar events take place in the initiation of translation in eukaryotic cells, but there are some important differences. In bacterial cells, sequences in 16S rRNA of the small ribosomal subunit bind to the Shine–Dalgarno sequence in mRNA. No analogous consensus sequence exists in eukaryotic mRNA. Instead, the cap at the 5′ end of eukaryotic mRNA plays a critical role in the initiation of translation. In a series of steps, the small subunit of the eukaryotic ribosome, initiation factors, and the initiator tRNA with its amino acid (Met-$tRNA_i^{Met}$) form an initiation complex that recognizes the cap and binds there. The initiation complex then moves along (scans) the mRNA until it locates the first AUG codon. The identification of the start codon is facilitated by the presence of a consensus sequence (called the Kozak sequence) that surrounds the start codon:



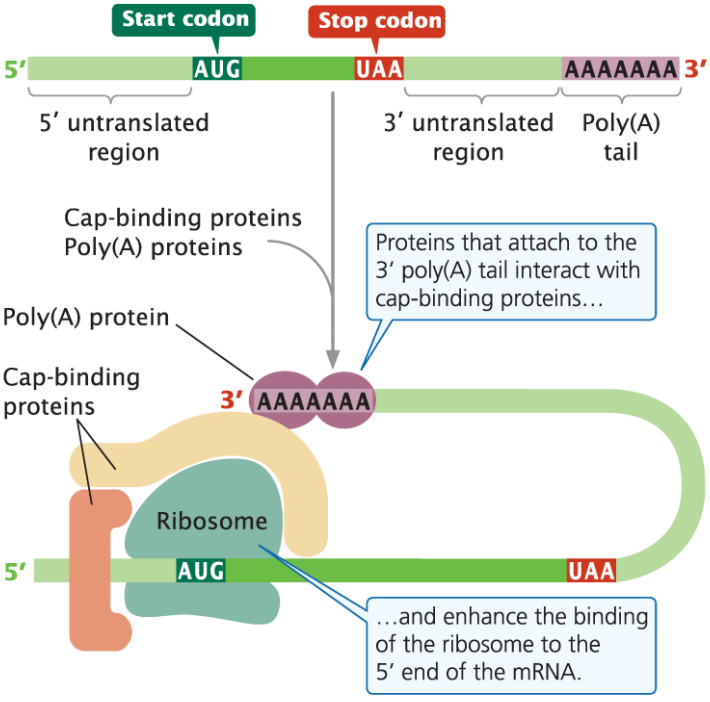
Once the initiation codon is reached, scanning ceases and the anticodon on initiator tRNA pairs with the start codon on the mRNA. Initiation factors are then released, and the large subunit of the ribosome joins the small subunit to create the functional ribosome.

Another important difference is that eukaryotic initiation requires at least 12 initiation factors. Some of these factors keep the ribosomal subunits separated, just as IF-3 does in bacterial cells. Others recognize the 5′ cap on the mRNA and allow the small ribosomal subunit to bind there. Still others possess RNA helicase activity, which is used to unwind secondary structures that may exist in the 5′ untranslated region of the mRNA, allowing the small subunit to move down the mRNA until the initiation codon is reached. Other initiation factors help bring Met-tRNA$_i^{Met}$ to the initiation complex.

In eukaryotes, the 5′ cap is initially bound by several proteins, one of which is the **cap-binding complex (CBC)**. The CBC aids in exporting the mRNA from the nucleus and then promotes the "pioneer," or initial, round of translation in the cytoplasm. This first round of translation plays an important role in checking for errors in the mRNA (see Messenger RNA Surveillance in Section 15.4). After the pioneer round of translation, the CBC is replaced by eukaryotic initiation factor 4E (eIF-4E), which promotes continued translation of the mRNA.

The poly(A) tail at the 3′ end of eukaryotic mRNA also plays a role in the initiation of translation. During initiation, proteins that attach to

the poly(A) tail interact with proteins that bind to the 5′ cap, enhancing the binding of the small ribosomal subunit to the 5′ end of the mRNA. This interaction indicates that the 3′ end of mRNA bends over and associates with the 5′ cap during the initiation of translation, forming a circular structure known as a closed loop (Figure 15.18).



15.18 The poly(A) tail of eukaryotic mRNA plays a role in the initiation of translation.

Description

The first part of the diagram at the top, shows 5 prime to 3 prime end m R N A strand. The start codon A U G is present near 5 prime end of the strand and the region between 5 prime end and the start codon represents the 5 prime untranslated region. Moving forward from left to the right stop codon U A A is present at the center of the strand followed by nucleotides A, A, A, A, A, A, A present at terminal 3 prime end which represents the poly

(A) tail. The region from stop codon to the initial point of poly (A) tail represents the 3 prime untranslated region.

An arrow from the strand is pointing downwards that represents the addition of cap-binding proteins poly (A) proteins.

The second part of the illustration shows loop of 5 prime to 3 prime end m R N A strand. The 3 prime end of the strand consists of poly (A) protein with A, A, A, A, A, A, A nucleotides. A caption pointing to the protein reads, Proteins that attach to the 3 prime poly (A) tail interact with cap-binding proteins and enhance the binding of the ribosome to the 5 prime end of the m R N A.

Towards the 5 prime end the large subunit of the ribosome is sitting on the small subunit and a start codon A U G is present at their center. One cap-binding protein is present at the top and one on the left of the ribosome. To the right of the ribosome, a stop codon U A A is present.

A few eukaryotic mRNAs contain internal ribosome entry sites, where ribosomes can bind directly without first attaching to the 5′ cap. Furthermore, some uncapped mRNAs are translated through the binding of initiation factors and ribosomes to modified adenine nucleotides (N^6-methyladenosine) in the mRNA.

Recent research that maps where ribosomes initiate translation on the mRNA has revealed surprising variation in translation initiation sites. Ribosomes frequently initiate translation at CUG and other non-AUG codons. In addition, a number of eukaryotic genes have short reading frames in the 5′ UTR of the mRNA upstream of the standard AUG start site (called upstream open reading frames, or uORFs) that may be translated into proteins, which then affects translation of downstream genes.

CONCEPTS

In the initiation of translation in bacterial cells, the small ribosomal subunit attaches to mRNA, and charged initiator tRNA attaches to the initiation codon. This process requires several initiation factors (IF-1, IF-2, and IF-3) and GTP. In the final step, the large ribosomal subunit joins the initiation complex.

✔ CONCEPT CHECK 7

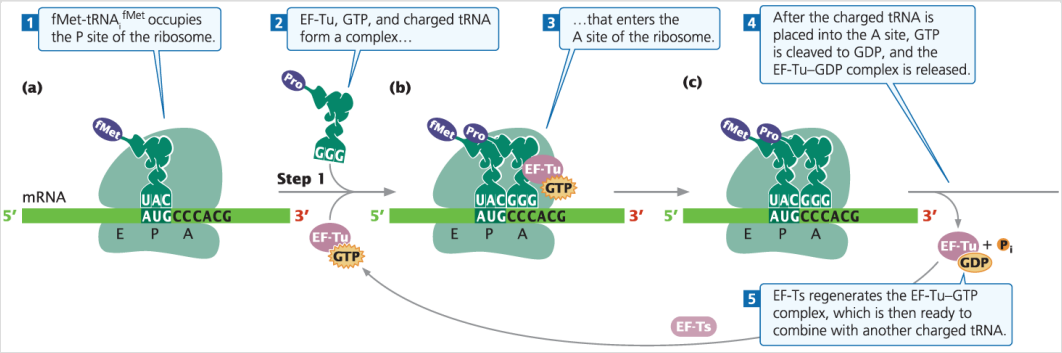
During the initiation of translation in bacteria, the small ribosomal subunit binds to which consensus sequence?

Elongation

The next stage in protein synthesis is elongation, in which amino acids are joined to create a polypeptide chain. Elongation requires (1) the 70S initiation complex just described, (2) tRNAs charged with their amino acids, (3) several elongation factors, and (4) GTP.

A ribosome has three sites that can be occupied by tRNAs: the **aminoacyl (A) site**, the **peptidyl (P) site**, and the **exit (E), site** (**Figure 15.19a**). The initiator tRNA immediately occupies the P site (the only site to which the fMet-$tRNA_i^{fMet}$ is able to bind), but all other tRNAs first enter the A site. At the end of initiation, the ribosome is attached to the mRNA, and fMet-$tRNA_i^{fMet}$ is positioned over the

AUG start codon in the P site; the adjacent A site is unoccupied (see Figure 15.19a).

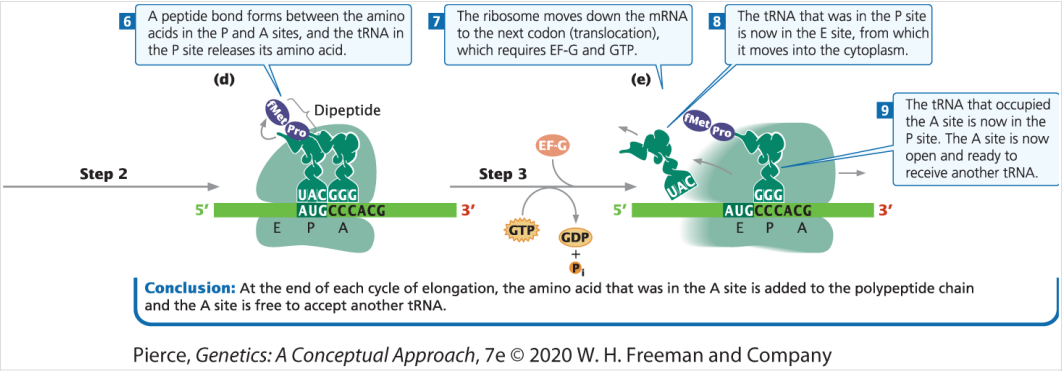


Description

Part a on the left starts with 5 prime to 3 prime ends of an m R N A strand where a ribosome with three binding sites E, P, and A is present at the center of the strand. A codon A U G is binding on the site P of the ribosome. A t R N A with anticodon U A C is attached with amino acid f methionine and sits on the codon at site P. A caption reads, f M e t-t R N A i, superscript f M e t occupies the P site of the ribosome.

A forward arrow labeled step 1 leads to part b and represents the addition of a t R N A with anticodon G G G attached to amino acid proline and elongation factor T u (E F-T u) attached to G T P.

Another caption reads, E F-T u, G T P, and charged t R N A form a complex that enters the A site of the ribosome. Part b in the center shows E F-T u, G T P, and charged t R N A complex sitting on site A of ribosome that has codon C C C. A forward arrow leads to part c on the right, which shows dissociation of G T P in to G D P, and release of the E F-T u–G D P complex. Next caption reads, After the charged t R N A is placed into the A site, G T P is cleaved to G D P, and the E F-T u–G D P complex is released. Last caption reads, E F-T s regenerates the E F-T u–G T P complex, which is then ready to combine with another charged t R N A. An arrow emerges from E F-T u and extends to G T P which is attached to E F-T u from step 1. This arrow shows E F-T s written over it.



15.19 The elongation stage of translation comprises three steps.

Description

A forward arrow labeled step 2 leads to part d on the right. Part d represents the formation of a dipeptide bond between the amino acids f methionine and proline that are attached to t R N A's in the P and A sites respectively. A caption reads, A peptide bond forms between the amino acids in the P and A sites, and the t R N A in the P site releases its amino acid. Another forward arrow labeled step 3 leads to part e and represents the addition of elongation factor G (E F- G) and hydrolysation of G T P in to G D P and P i.

Part e on the right shows relocation of ribosome on the 5 prime to 3 prime m R N A strand, towards the 3 prime end. A caption reads, The ribosome moves down the m R N A to the next codon (translocation), which requires E F-G and G T P. As a result of relocation, t R N A with anticodon U A C is released. Another caption reads, The t R N A that was in the P site is now in the E site, from which it moves into the cytoplasm. Further codon A U G is shifted from site P to site E, and t R N A with anticodon G G G and attached to amino acids f methionine and proline; is shifted from site A to site P with codon C C C. The last caption reads, The t R N A that occupied the A site is now in the P site. The A site is now open and ready to receive another t R N A.

A caption below reads, conclusion: At the end of each cycle of elongation, the amino acid that was in the A site is added to the polypeptide chain and the A site is free to accept another t R N A.

Elongation takes place in three steps. In the first step (Figure 15.19b), a charged tRNA binds to the A site. This binding takes place

when **elongation factor Tu (EF-Tu)** joins with GTP and then with a charged tRNA to form a three-part complex. This complex enters the A site of the ribosome, where the anticodon on the tRNA pairs with the codon on the mRNA. Once the charged tRNA is in the A site, GTP is cleaved to form GDP, and the EF-Tu–GDP complex is released (**Figure 15.19c**). **Elongation factor Ts (EF-Ts)** regenerates EF-Tu–GDP to EF-Tu–GTP. In eukaryotic cells, a similar set of reactions delivers a charged tRNA to the A site.

The second step of elongation is the formation of a peptide bond between the amino acids that are attached to tRNAs in the P and A sites (**Figure 15.19d**). The formation of this peptide bond releases the amino acid in the P site from its tRNA. Peptide-bond formation occurs within the large ribosomal subunit. The catalytic activity that creates the peptide bond is a property of one of the rRNA components of the large subunit (the 23S rRNA in bacteria, the 28S RNA in eukaryotes); this rRNA acts as a ribozyme (see p. 372 in Chapter 13).

The third step in elongation is **translocation** (**Figure 15.19e**), the movement of the ribosome down the mRNA in the $5' \rightarrow 3'$ direction. This step, which positions the ribosome over the next codon, requires **elongation factor G (EF-G)** and the hydrolysis of GTP to GDP. Because the tRNAs in the P and A sites are still attached to the mRNA by codon–anticodon pairing, they do not move with the ribosome as it translocates. Consequently, the ribosome shifts so that the tRNA that previously occupied the P site now occupies the E

site, from which it then moves into the cytoplasm, where it can be recharged with another amino acid. Translocation also causes the tRNA that occupied the A site (which is attached to the growing polypeptide chain) to occupy the P site, leaving the A site open. Thus, the progress of each tRNA through the ribosome in the course of elongation can be summarized as follows: cytoplasm → A site → P site → E site → cytoplasm. As stated earlier, the initiator tRNA is an exception: it attaches directly to the P site and never occupies the A site.

After translocation, the A site of the ribosome is empty and ready to receive the tRNA specified by the next codon. The elongation cycle (see Figure 15.19b–e) repeats itself: a charged tRNA and its amino acid occupy the A site, a peptide bond is formed between the amino acids in the A and P sites, and the ribosome translocates to the next codon. Throughout the cycle, the polypeptide chain remains attached to the tRNA in the P site. Another protein, called elongation factor P (EF-P), enhances the translation of proteins that contain consecutive copies of the amino acid proline. If EF-P is absent, ribosomes often stall during the translation of such polyproline-containing proteins.

Messenger RNAs, although single stranded, often contain secondary structures formed by the pairing of complementary bases on different parts of the mRNA molecule (see Figure 13.1b). As the ribosome moves along the mRNA, these secondary structures are unwound by helicase activity located in the small ribosomal subunit.

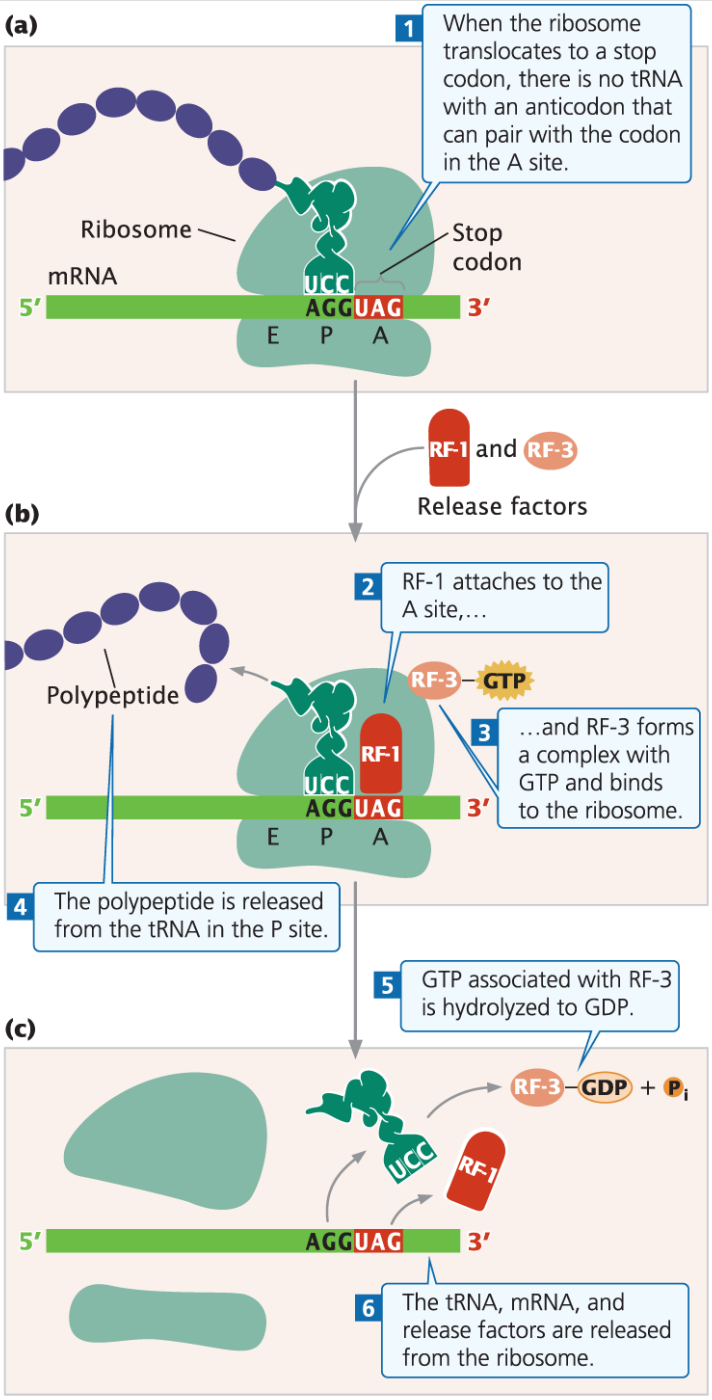
Researchers have developed methods for following a single ribosome as it translates individual codons of an mRNA molecule. These studies have revealed that translation does not take place in a smooth, continuous fashion. Each translocation of the ribosome typically requires less than a tenth of a second, but sometimes there are distinct pauses, often lasting a few seconds, between translocations. Thus, translation takes place in a series of quick translocations interrupted by brief pauses. In addition to the short pauses between translocation events, translation may be interrupted by longer pauses—lasting from 1 to 2 minutes—which may play a role in regulating the process of translation.

Elongation in eukaryotic cells takes place in a manner similar to that in bacteria. Eukaryotes possess at least three elongation factors, one of which also acts in initiation and termination. Another of these elongation factors, called eukaryotic elongation factor 2 (eEF-2), is the target of a toxin produced by the bacterium that causes diphtheria, a disease that until recently was a leading killer of children. The diphtheria toxin inhibits eEF-2, preventing the translocation of the ribosome along the mRNA, and protein synthesis ceases.

Termination

Protein synthesis ends when the ribosome translocates to a termination codon. Because there are no tRNAs with anticodons

complementary to the termination codons, no tRNA enters the A site of the ribosome (**Figure 15.20a**). Instead, proteins called **release factors** bind to the ribosome (**Figure 15.20b**). *Escherichia coli* has three release factors: **RF-1**, **RF-2**, and **RF-3**. Release factor 1 binds to the termination codons UAA and UAG, and RF-2 binds to UGA and UAA. The binding of RF-1 or RF-2 to the A site of the ribosome promotes the cleavage of the tRNA in the P site from the polypeptide chain and the release of the polypeptide. Release factor 3 forms a complex with GTP and binds to the ribosome. This complex brings about a conformational change in the ribosome, which releases RF-1 or RF-2 from the A site and causes the tRNA in the P site to move to the E site; in the process, GTP is hydrolyzed to GDP. Additional factors help bring about the release of the tRNA from the P site, the release of the mRNA from the ribosome, and the dissociation of the ribosome (**Figure 15.20c**).



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15.20 Translation ends when a stop codon is encountered. Because UAG is the termination codon in this illustration, the release factor is RF-1.

Description

In part (a) at the top, a 5 prime to 3 prime m R N A strand is passing through the separating section of large and small subunits of a ribosome. Three t R N A binding sites located at R N A sequence from left to right are E, P, and A. A t R N A sequence A G G is attached at the site P and an anti codon U C C, attached with a polypeptide chain is binding to t R N A sequence A G G. A stop codon U A C is attached at binding site A. A caption reads, When the ribosome translocates to a stop codon, there is no t R N A with an anticodon that can pair with the codon in the A site.

In part (b) below, a release factor R F-1 is binding above the stop codon at the site A. Another caption reads, R F-1 attaches to the A site and R F-3 forms a complex with G T P and binds to the ribosome. Another release factor R F-3 forms a complex with G T P and binds at the upper edge of the ribosome to the right of site A. The polypeptide chain is separated from the anti codon U C C and the next caption reads, The polypeptide is released from the t R N A in the P site.

In part (c) below, G T P attached with R F-3 is hydrolyzed in to G D P and releases a P subscript i molecule. A caption reads G T P associated with R F-3 is hydrolyzed to G D P. The ribosome's small and large units split releasing all the bindings of t R N A, m R N A, and binding sites. The last caption reads, The t R N A, m R N A, and release factors are released from the ribosome.

It is important to note that the termination codon is not located at the 3′ end of the mRNA; rather, the termination codon is followed by a number of nucleotides that constitute the 3′ untranslated region (UTR) of the mRNA. The 3′ UTR often contains sequences that affect the stability of the mRNA and influence whether translation takes place (see Chapter 17).

THINK-PAIR-SHARE Question 8

CONCEPTS

Elongation consists of three steps: (1) a charged tRNA enters the A site, (2) a peptide bond is created between amino acids in the A and P sites, and (3) the ribosome translocates to the next codon. Elongation requires several elongation factors and GTP.

✔ CONCEPT CHECK 8

In elongation, the creation of peptide bonds between amino acids is catalyzed by

a. rRNA.
b. a protein in the small ribosomal subunit.
c. a protein in the large ribosomal subunit.
d. tRNA.

Research shows that some bacterial ribosomes engage in a type of proofreading, similar to the proofreading that DNA polymerases perform during replication. After translocation, the ribosome checks the interaction between the mRNA and the tRNA in the P site. If the wrong tRNA was added, the alignment between the mRNA and the tRNA will be incorrect, which will trigger premature termination of translation. Evidence suggests that an important function of RF-3 is to help bring about termination of translation when the wrong tRNA has been used. You can explore the process of bacterial translation by examining the consequences of various mutations in the coding region of a gene in (A) **Animation 15.1**.

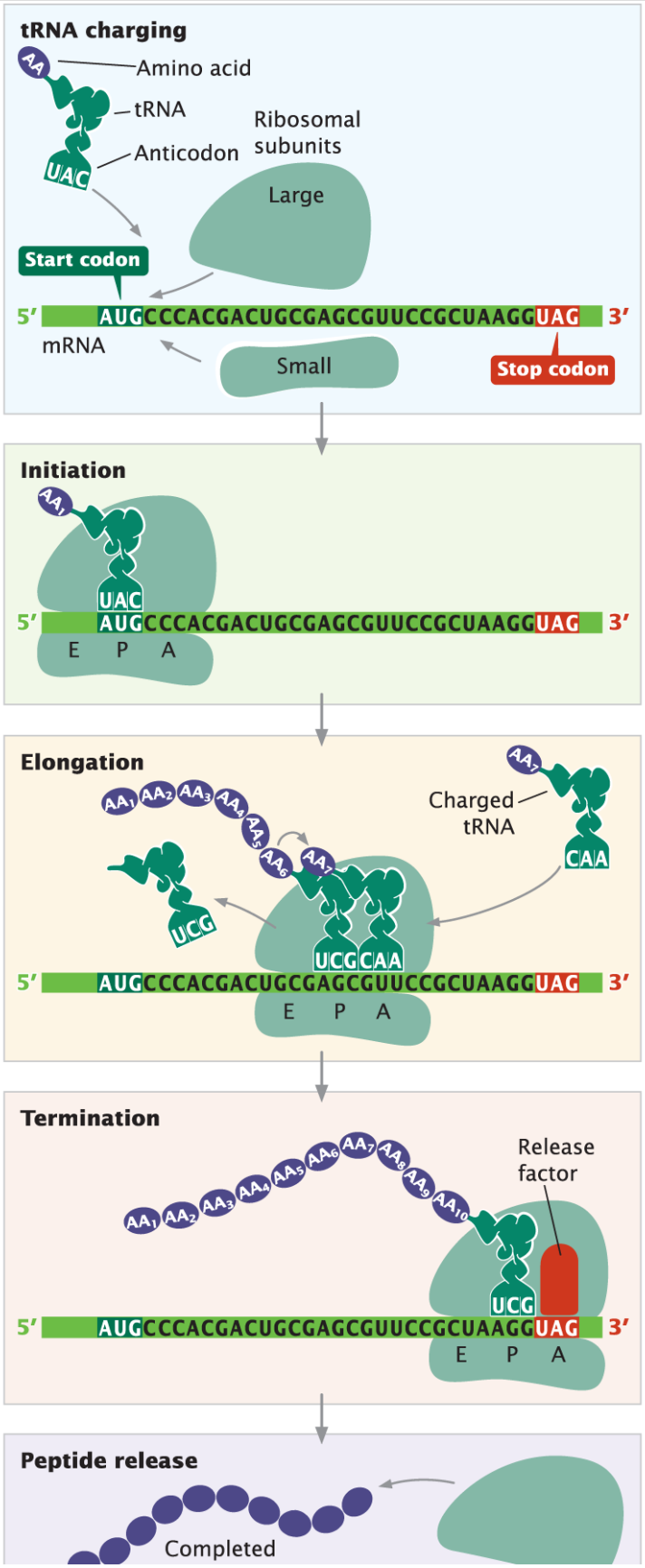
Translation in eukaryotic cells terminates in a similar way, except that there are two release factors: eRF-1, which recognizes all three termination codons, and eRF-2, which binds GTP and stimulates the release of the polypeptide from the ribosome. No translational

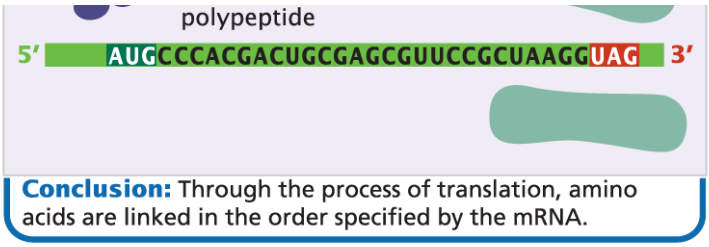
proofreading, such as that stimulated by RF-3 in bacteria, has been observed in eukaryotic cells. **› TRY PROBLEMS 30 AND 34**

CONCEPTS

Translation ends when the ribosome reaches a termination codon. Release factors bind to the termination codon, causing the release of the polypeptide from the last tRNA, of the tRNA from the ribosome, and of the mRNA from the ribosome.

The overall process of protein synthesis, including tRNA charging, initiation, elongation, and termination, is summarized in Figure 15.21. The components taking part in this process are listed in Table 15.4.


tRNA charging
AA
Amino acid
tRNA
Anticodon
UAC
Ribosomal subunits
Large
Start codon
5′ AUGCCCACGACUGCGAGCGUUCCGCUAAGGUAG 3′
mRNA
Small
Stop codon
Initiation
AA1
UAC
5′ AUGCCCACGACUGCGAGCGUUCCGCUAAGGUAG 3′
E P A
Elongation
AA1 AA2 AA3 AA4 AA5 AA6 AA7
Charged tRNA
CAA
UCG
UCG CAA
5′ AUGCCCACGACUGCGAGCGUUCCGCUAAGGUAG 3′
E P A
Termination
AA1 AA2 AA3 AA4 AA5 AA6 AA7 AA8 AA9 AA10
Release factor
UCG
5′ AUGCCCACGACUGCGAGCGUUCCGCUAAGGUAG 3′
E P A
Peptide release
Completed



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15.21 Translation requires tRNA charging, initiation, elongation, and termination. In the process of translation, amino acids are linked together in the order specified by mRNA to create a polypeptide chain. A number of initiation, elongation, and release factors take part in the process, and energy is supplied by ATP and GTP.

Description

Each part of the diagram represents a different step in the translation process.

In the first part labeled t R N A charging at the top, the small and large ribosomal subunits are separated by a 5 prime to 3 prime m R N A strand and a t R N A attached with an amino acid A A and an anti codon U A C approaches towards the m R N A strand. A start codon A U G represented by the color green is present towards 5 prime end while a stop codon U A G represented by the color red is present towards 3 prime end of the m R N A strand.

In the second part below labeled initiation, the gap between ribosomal subunits is reduced and both the subunits are located towards the 5 prime end of m R N A strand. The ribosome has three binding sites E, P, and A from left to the right. The t R N A attached with the amino acid binds with the start codon at the site P.

In the third part below labeled elongation, ribosomal subunits are located near to the center of 5 prime to 3 prime end of m R N A sequence. From the right side of the diagram, a charged t R N A, C A A attached with an amino acid sequence A A binds to the m R N A sequence at the site A. A t R N A sequence U C G attaches to a growing chain of amino acids and binds with the ribosomal unit at site P. An arrow from site E of the ribosome points towards t R N A sequence U C G located at the left of the diagram.

In the fourth part below labeled termination, the ribosome is shifted towards the 3 prime end of the m R N A strand where the release factor binds at the stop codon sequence U A

G at site A while a t R N A sequence U C G attached with a growing chain of amino acids binds ribosome at site P.

In the fifth part below labeled peptide release, the ribosomal units, small and large, are released at the 3 prime end of the m R N A sequence and a completed polypeptide dissociates from the ribosome.

A caption below reads, conclusion: Through the process of translation, amino acids are linked in the order specified by the m R N A.

TABLE 15.4 Components required for protein synthesis in bacterial cells

Stage	Component	Function
tRNA charging	Amino acids	Building blocks of proteins
	tRNAs	Deliver amino acids to ribosomes
	Aminoacyl-tRNA synthetases	Attach amino acids to tRNAs
	ATP	Provides energy for binding amino acids to tRNAs
Initiation	mRNA	Carries coding instructions
	fMet-	Provides first amino acid

	$tRNA_i^{fMet}$	in peptide
	30S ribosomal subunit	Attaches to mRNA
	50S ribosomal subunit	Stabilizes tRNAs and amino acids
	Initiation factor 1	Enhances dissociation of large and small subunits of ribosome
	Initiation factor 2	Binds GTP; delivers fMet-$tRNA_i^{fMet}$ to initiation codon
	Initiation factor 3	Binds to 30S subunit and prevents association with 50S subunit
Elongation	70S initiation complex	Functional ribosome with A, P, and E sites where protein synthesis takes place
	Charged tRNAs	Bring amino acids to ribosome and help

		assemble them in order specified by mRNA
	Elongation factor Tu	Binds GTP and charged tRNA; delivers charged tRNA to A site
	Elongation factor Ts	Regenerates active elongation factor Tu
	Elongation factor G	Stimulates translocation of ribosome to next codon
	GTP	Provides energy
	23S rRNA in large ribosomal subunit	Creates peptide bond between amino acids in A site and P site
Termination	Release factors 1, 2, and 3	Bind to ribosome when stop codon is reached and terminate translation

CONNECTING CONCEPTS

A Comparison of Bacterial and Eukaryotic Translation

We have now considered the process of translation in bacterial cells and noted some distinctive differences that exist in eukaryotic cells. Let's reflect on some of the important

similarities and differences between protein synthesis in bacterial and in eukaryotic cells.

First, we should emphasize that the genetic code of bacterial and eukaryotic cells is virtually identical; the only difference is in the amino acid specified by the initiation codon. In bacterial cells, the initiation codon AUG encodes a modified type of methionine, *N*-formylmethionine, whereas in eukaryotic cells, AUG encodes unformylated methionine. One consequence of the fact that bacteria and eukaryotes use the same genetic code is that eukaryotic genes can be translated in bacterial systems, and vice versa; this feature makes genetic engineering possible, as we will see in Chapter 19.

Another difference is that transcription and translation take place simultaneously in bacterial cells, but the nuclear envelope separates these processes in eukaryotic cells. The physical separation of transcription and translation has important implications for the control of gene expression in eukaryotes, which we will consider in Chapter 17, and it allows for extensive modification of eukaryotic mRNAs, as discussed in Chapter 14.

Yet another difference is that mRNA in bacterial cells is short-lived, typically lasting only a few minutes, but mRNA in eukaryotic cells can last hours or days. The 5′ cap and 3′ poly(A) tail found on eukaryotic mRNAs add to their stability (see Chapter 14).

In both bacterial and eukaryotic cells, aminoacyl-tRNA synthetases attach amino acids to their appropriate tRNAs, and the chemical process is the same.

There are significant differences in the sizes and compositions of bacterial and eukaryotic ribosomal subunits. For example, the large subunit of the eukaryotic ribosome contains three rRNAs, whereas that of the bacterial ribosome contains only two (see Table 14.3). These differences allow antibiotics and other substances to inhibit bacterial translation while having no effect on the translation of eukaryotic nuclear genes, as we will see in Section 15.4.

Other fundamental differences lie in the process of initiation. In bacterial cells, the small ribosomal subunit attaches directly to the region surrounding the start codon through base pairing between the Shine–Dalgarno sequence in the 5′ UTR of the mRNA and a sequence at the 3′ end of the 16S rRNA. In contrast, the small subunit of a eukaryotic ribosome first binds to proteins attached to the 5′ cap on mRNA and then migrates down the mRNA, scanning the sequence until it encounters the first initiation codon. Additionally, a larger number of initiation factors take part in eukaryotic initiation than in bacterial initiation.

Elongation and termination are similar in bacterial and eukaryotic cells, although different elongation and termination factors are used. In both types of organisms, mRNAs are translated multiple times and are simultaneously attached to several ribosomes, forming polyribosomes, as discussed in Section 15.4.

Less is known about the process of translation in archaea, but they appear to possess a mixture of bacterial and eukaryotic features. Because archaea lack nuclear membranes, transcription and translation take place simultaneously, just as they do in bacterial cells. Archaea use unformylated methionine as the initiator amino acid, a characteristic of eukaryotic translation. Some of the initiation and release factors in archaea are similar to those found in bacteria, whereas others are similar to those found in eukaryotes. Finally, some of the antibiotics that inhibit translation in bacteria have no effect on translation in archaea, providing further evidence of the fundamental differences between bacteria and archaea.

15.4 Additional Properties of RNA and Ribosomes Affect Protein Synthesis

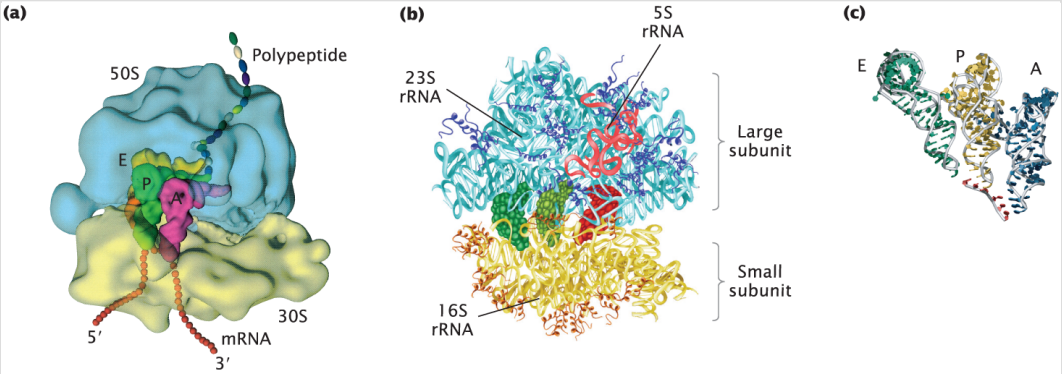
Now that we have considered the process of translation in some detail, let's examine some additional aspects of protein synthesis and the translation machinery.

The Three-Dimensional Structure of the Ribosome

The central role of the ribosome in protein synthesis was recognized in the 1950s, and numerous aspects of its structure have been studied since then. Nevertheless, many aspects remained a mystery until detailed, three-dimensional reconstructions were completed. In 2009, the Nobel Prize in chemistry was awarded to Venkatraman Ramakrishnan, Thomas Steitz, and Ada Yonath for their research on the molecular structure of the ribosome.

Figure 15.22a shows a model of the *E. coli* ribosome at low resolution. A high-resolution image of the bacterial ribosome structure as determined by X-ray crystallography is represented in the model depicted in Figure 15.22b. An mRNA molecule is bound to the small ribosomal subunit, and tRNAs are located in the A, P,

and E sites (**Figure 15.22c**) that bridge the small and large subunits (see Figure 15.22a). Initiation factors 1 and 3 bind to sites on the outside of the small subunit. EF-Tu, EF-G, and other factors complexed with GTP interact with a *factor-binding center*. High-resolution crystallographic images provide information indicating that a *decoding center* resides in the small subunit (the decoding center cannot be seen in Figure 15.22b). This center senses the fit between the codon on the mRNA and the anticodon on the incoming charged tRNA. Only tRNAs with the correct anticodon are bound tightly by the ribosome. These structural analyses indicate that peptide-bond formation occurs within the large ribosomal subunit and is carried out by the 23S rRNA molecule. These analyses also reveal that a tunnel connects the site of peptide-bond formation with the back of the ribosome; the growing polypeptide chain passes through this tunnel to the outside of the ribosome. The tunnel can accommodate about 35 amino acids of the growing polypeptide chain.



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Part b: EMILY CIOSEK/Science Source.

15.22 Structure of the ribosome. (a) Low-resolution model of the ribosome, showing the A, P, and E sites where tRNAs, the mRNA, and the growing

polypeptide chain reside. (b) High-resolution model of the ribosome. The tRNA in the E site is forest green, the tRNA in the P site is yellowish-green, and the tRNA in the A site is red. The 16S rRNA is shown in yellow, the 23S rRNA in cyan, and the 5S rRNA in red. Small ribosomal subunit proteins are shown in orange, whereas large ribosomal subunit proteins are shown in purple. (c) Positions of tRNAs in E, P, and A sites of the ribosome shown in part *b*.

Description
Part a on the left shows a low-resolution model of the ribosome. The ribosome has a 50 S large subunit sitting on the 30 S small subunit of the ribosome. The ribosome shows A, P, and E sites at the center where a 5 prime to 3 prime end m R N A and a growing polypeptide chain reside.

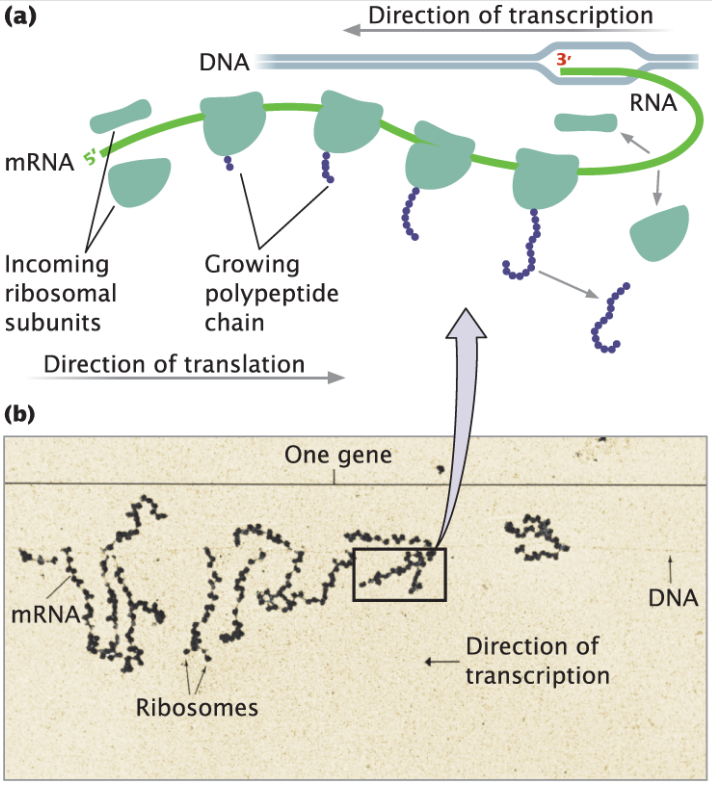
Part b in the center shows a high-resolution model of the ribosome as a ribbon-structure. The large subunit of the ribosome has 23 S r R N A and the 5 S r R N A. The small subunit of the ribosome has 16 S r R N A.

Part c on the right shows double helical structure of the E, P, A binding sites of ribosome.

Polyribosomes

In both prokaryotic and eukaryotic cells, mRNA molecules are translated simultaneously by multiple ribosomes (Figure 15.23). The resulting structure—an mRNA with several ribosomes attached—is called a **polyribosome** (or often just a *polysome*). Each ribosome successively attaches to the ribosome-binding site at the $5'$ end of the mRNA and moves toward the $3'$ end; the polypeptide associated with each ribosome becomes progressively longer as the ribosome moves along the mRNA. In prokaryotic cells, transcription and

translation are simultaneous; multiple ribosomes may be attached to the 5′ end of the mRNA while transcription is still taking place at the 3′ end, as shown in Figure 15.23. Ribosomes translating the mRNA interact with and influence transcription carried out by RNA polymerase (see Chapter 16); together, RNA polymerase and the first ribosome form a complex that has been called the expressome. In eukaryotes, transcription and translation are separated in time and space; transcription takes place in the nucleus, and translation takes place in the cytoplasm.



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Part b: From O. L. Miller, Jr., B. A. Hamkalo, and C. A. Thomas, Jr., "Visualization of Bacterial Genes in Action," *Science* 169: 392, 1970. Reprinted with permission from AAAS, permission conveyed through Copyright Clearance Center, Inc.

15.23 An mRNA molecule may be translated simultaneously by several ribosomes. (a) Four ribosomes are translating an mRNA molecule; the ribosomes move from the $5'$ end to the $3'$ end of the mRNA. (b) In this electron micrograph, the long horizontal filament is DNA, the dark-staining spheres are polyribosomes, and the thin filaments connecting the ribosomes are mRNAs. Transcription of the DNA is proceeding from right to left; the mRNAs on the right are shorter than those on the left. Each mRNA is being translated by multiple ribosomes.

Description

In part (a) at the top, a spiral m R N A strand starts with 5 prime and ends at 3 prime which is attached to a double stranded D N A sequence and an arrow above the D N A sequence is moving from right to left and a text on the arrow reads: Direction of transcription.

5 prime of the m R N A sequence separates from small and large subunits of the ribosome located above and below the strand followed by ribosomes with growing polypeptide chain. An arrow from the 5 prime end moves towards right and the text on the arrow reads: Direction of translation. A ribosome nearest the D N A splits into its small and large subunits dissociating the polypeptide chain towards 3 prime end.

In part (b) below, an electron micrograph starts from left to right where several spherical ribosomes are connected by filaments of m R N A's. Proceeding right, a rectangular box contains ribosomes attached with m R N A's and an arrow from the box points above towards ribosome with growing polypeptide chain of part (a) of the illustration. Moving forward, short filaments of m R N A's are binding ribosomes and in the right side of the illustration D N A is formed. An arrow at the bottom of micrograph moving from right to left reads: Direction of transcription. Text above the micrograph reads: One gene.

CONCEPTS

In both prokaryotic and eukaryotic cells, multiple ribosomes may translate a single mRNA molecule simultaneously, generating a structure called a polyribosome.

✔ CONCEPT CHECK 9

In a polyribosome, the polypeptides associated with which ribosomes will be the longest?

a. Those at the 5′ end of mRNA
b. Those at the 3′ end of mRNA
c. Those in the middle of mRNA
d. All polypeptides will be the same length.

Messenger RNA Surveillance

The accurate transfer of genetic information from one generation to the next and from genotype to phenotype is critical for the proper development and functioning of an organism. Consequently, cells have evolved a number of quality-control mechanisms to ensure the accuracy of information transfer. Protein synthesis is no exception: several mechanisms, collectively termed **mRNA surveillance**, exist to detect and deal with errors in mRNAs that may create problems in the course of translation. These mechanisms keep the cell from wasting resources translating aberrant mRNAs and prevent the production of truncated proteins, which may be toxic to the cell.

NONSENSE-MEDIATED mRNA DECAY

A common type of mutation is the alteration of a codon that specifies an amino acid to become a termination codon (called a nonsense mutation, see Chapter 18). A nonsense mutation does not affect transcription, but translation ends prematurely when the

termination codon is encountered. The resulting protein is truncated and often nonfunctional. Nonsense mutations often arise in eukaryotic transcription when one or more exons are skipped or improperly spliced. Improper splicing leads to the deletion or addition of nucleotides in the mRNA, which alters the reading frame and often introduces premature termination codons.

To prevent the synthesis of aberrant proteins resulting from nonsense mutations, eukaryotic cells have evolved a mechanism called **nonsense-mediated mRNA decay (NMD)**, which results in the rapid elimination of mRNA containing premature termination codons. The mechanism responsible for NMD is still poorly understood. In mammals, it appears to entail proteins that bind to exon–exon junctions. These exon-junction proteins may interact with enzymes that degrade the mRNA. One possibility is that the first ribosome to translate an mRNA (in the pioneer round of translation) removes the exon-junction proteins, thus protecting the mRNA from degradation. However, when the ribosome encounters a premature termination codon, the ribosome does not traverse the entire mRNA, and some of the exon-junction proteins are not removed, resulting in NMD. Nonsense-mediated mRNA decay does not occur in bacteria. ›

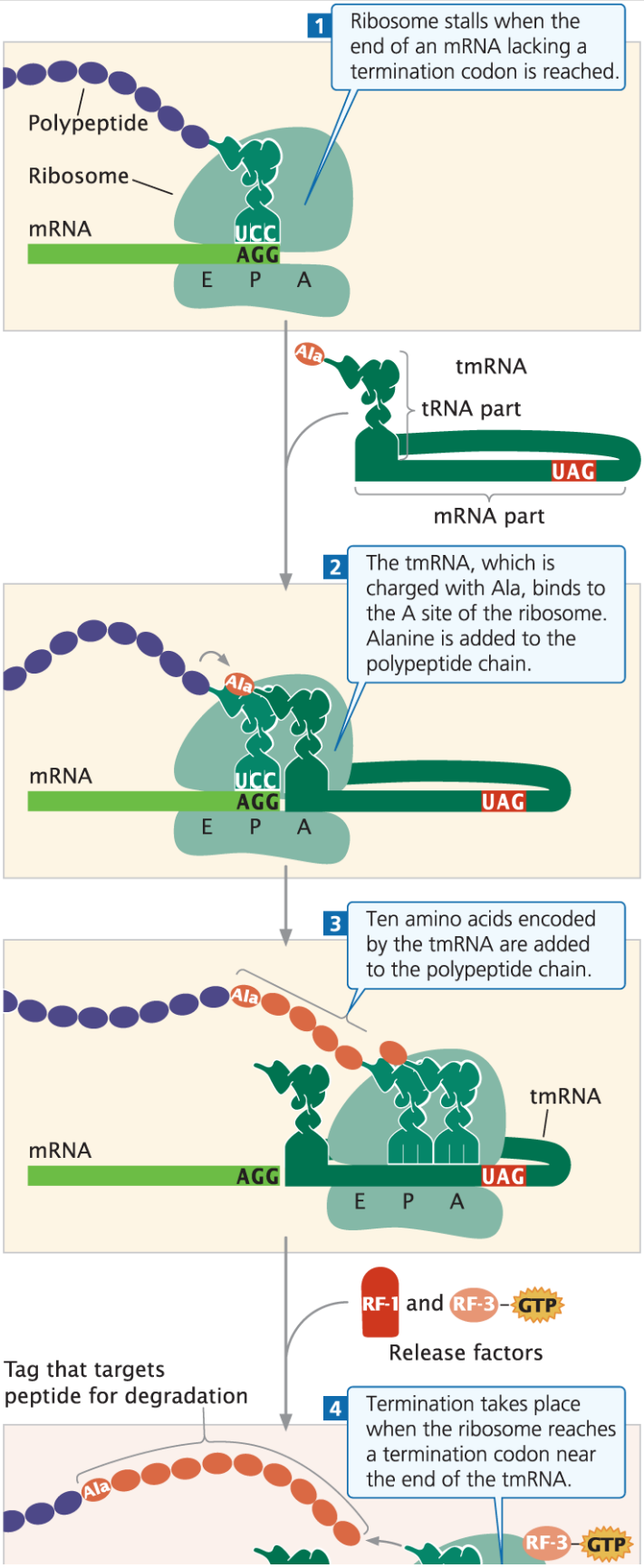
TRY PROBLEM 39

STALLED RIBOSOMES AND NONSTOP mRNAs

A problem that occasionally arises in translation is the stalling of a ribosome on mRNA before translation is terminated. This situation

can arise when a mutation in DNA changes a termination codon into a codon that specifies an amino acid. It can also arise when transcription terminates prematurely, producing a truncated mRNA lacking a termination codon. In these cases, the ribosome reaches the end of the mRNA without encountering a termination codon, whereupon it stalls, still attached to the mRNA. Attachment to the mRNA prevents the ribosome from being recycled for use on other mRNAs; if such occurrences are frequent, the result is a shortage of ribosomes that diminishes overall levels of protein synthesis.

Bacteria have evolved a kind of molecular tow truck called **transfer–messenger RNA (tmRNA)**, which removes stalled ribosomes. This RNA molecule has properties of both tRNA and mRNA; its tRNA component is normally charged with the amino acid alanine. When a ribosome becomes stalled on an mRNA, EF-Tu delivers the tmRNA to the ribosome's A site, where it acts as a surrogate tRNA (**Figure 15.24**). A peptide bond is created between the amino acid in the P site of the ribosome and the alanine (attached to tmRNA) now in the A site, transferring the polypeptide chain to the tmRNA and releasing the tRNA in the P site.


1
Ribosome stalls when the end of an mRNA lacking a termination codon is reached.
Polypeptide
Ribosome
mRNA
UCC
AGG
E P A
Ala
tmRNA
tRNA part
UAG
mRNA part
2
The tmRNA, which is charged with Ala, binds to the A site of the ribosome. Alanine is added to the polypeptide chain.
Ala
mRNA
UCC
AGG
UAG
E P A
3
Ten amino acids encoded by the tmRNA are added to the polypeptide chain.
Ala
tmRNA
mRNA
AGG
UAG
E P A
RF-1 and RF-3 GTP
Release factors
Tag that targets peptide for degradation
4
Termination takes place when the ribosome reaches a termination codon near the end of the tmRNA.
Ala
RF-3 GTP

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15.24 In bacteria, tmRNA allows stalled ribosomes to resume translation.

Description

The first part at the top shows a ribosome with binding sites E, P, and A placed at the right end of an m R N A strand and a growing polypeptide chain attached with t R N A-U C C, binding at site P of ribosome. The first caption reads, Ribosome stalls when the end of an m R N A lacking termination codon is reached.

In the second part below, t R N A portion of t m R N A is attached with alanine and binds to ribosome at site A in a manner that alanine forms complex with polypeptide chain attached to t R N A-U C C. The long tailed portion of t m R N A contains stop codon U A G. The second caption reads, The t m R N A, which is charged with A l a, binds to the A site of the ribosome. Alanine is added to the polypeptide chain.

In the third part below, alanine sequences are binding to polypeptide chain and a t- R N A is located in the left of site E of ribosome. The third caption reads, Ten amino acids encoded by the t m R N A are added to the polypeptide chain.

In the fourth part below, a release factor R F-1 is binding above the stop codon at the site A while another release factor R F-3 forms a complex with G T P and binds at the upper edge of the ribosome to the right of site A. The fourth caption reads, Termination takes place when the ribosome reaches a termination codon near the end of the t m R N A.

The amino acid chain that was added to the polypeptide chain is labeled: Tag that targets peptide for degradation.

The ribosome then resumes translation, switching from the original, aberrant mRNA to the mRNA part of tmRNA. It adds 10 amino acids encoded by the tmRNA and then reaches a termination codon at the

3′ end of the tmRNA, which terminates translation and releases the ribosome. The added amino acids act as a tag that targets the incomplete polypeptide chain for degradation. Some evidence suggests that the tmRNA also targets the aberrant mRNA for degradation. How stalled ribosomes are recognized by the tmRNA is not clear, but this method is efficient at recycling stalled ribosomes and eliminating abnormal proteins that result from truncated transcription. Some bacteria have an alternative method for rescuing stalled ribosomes that utilizes proteins called alternative ribosome-rescue factors (Arfs). An alternative ribosome-rescue factor called ArfA binds to the A site of the ribosome and substitutes for the missing stop codon by recruiting release factor 2, which then brings about termination of translation.

Eukaryotes have evolved a different mechanism to deal with mRNAs that are missing termination codons. Instead of restarting the stalled ribosome and degrading the abnormal protein that results, eukaryotic cells use a mechanism called **nonstop mRNA decay**, which results in the rapid degradation of abnormal mRNA. In this mechanism, the codon-free A site of the stalled ribosome is recognized by a special protein, which binds to the A site and recruits other proteins, which then degrade the mRNA from its 3′ end.

NO-GO DECAY

Another mRNA surveillance system found in eukaryotes is **no-go decay (NGD)**, which helps remove stalled ribosomes resulting from secondary structures in the mRNA, chemical damage to the mRNA, premature stop codons, and ribosomal defects. A series of proteins brings about termination, recycling of the ribosomes, and degradation of the mRNA.

CONCEPTS

Cells possess mRNA surveillance mechanisms to detect and eliminate mRNA molecules containing errors that may create problems in the course of translation.

Folding and Posttranslational Modifications of Proteins

The functions of many proteins depend critically on the proper folding of the polypeptide chain. Some proteins spontaneously fold into their correct shapes, but for others, correct folding may initially require the participation of other molecules, called **molecular chaperones**. Some molecular chaperones are associated with the ribosome and fold newly synthesized polypeptide chains as they emerge from the ribosome tunnel, in which case protein folding takes place as translation is ongoing.

Many proteins must be modified after translation to become functional. Proteins in both prokaryotic and eukaryotic cells often undergo alterations following translation, which are termed posttranslational modifications. A number of different types of modifications are possible. Some proteins are synthesized as part of larger precursor proteins that must be cleaved and trimmed by enzymes before the proteins can become functional. As mentioned earlier, the formyl group, or the entire methionine, may be removed from the amino end of a protein. Some proteins require the attachment of carbohydrates for activation. Amino acids within a protein may be modified: phosphates, carboxyl groups, and methyl groups are added to some amino acids. In eukaryotic cells, the amino end of a protein is often acetylated after translation.

A common posttranslational modification in eukaryotes is the attachment of a protein called ubiquitin, which targets the protein for degradation. Another modification of some proteins is the removal of 15 to 30 amino acids, called the **signal sequence**, at the amino end of the protein. The signal sequence helps direct a protein to a specific location within the cell, after which the sequence is removed by special enzymes.

THINK-PAIR-SHARE Question 9

CONCEPTS

Many proteins undergo posttranslational modifications after their synthesis.

Translation and Antibiotics

Antibiotics are drugs that kill bacteria. To make an effective antibiotic, not just any poison will do: the trick is to kill the bacteria without harming the patient.

Translation is frequently the target of antibiotics because it is essential to all living organisms and differs significantly between bacterial and eukaryotic cells. A number of antibiotics bind selectively to bacterial ribosomes and inhibit specific steps in translation but do not affect eukaryotic ribosomes. Tetracyclines, for instance, are a class of antibiotics that bind to the A site of a bacterial ribosome and block the entry of charged tRNAs, yet they have no effect on eukaryotic ribosomes. Neomycin binds to the ribosome near the A site and induces translational errors, probably by causing mistakes in the binding of charged tRNAs to the A site. Chloramphenicol binds to the large ribosomal subunit and blocks peptide-bond formation. Streptomycin binds to the small ribosomal subunit and inhibits initiation, and erythromycin blocks translocation. Although chloramphenicol and streptomycin are potent inhibitors of translation in bacteria, they do not inhibit translation in archaea.

The three-dimensional structure of puromycin resembles that of the 3′ end of a charged tRNA, which permits puromycin to enter the A site of a ribosome efficiently and inhibit the entry of tRNAs. A peptide bond can form between the puromycin molecule in the A site and an amino acid on the tRNA in the P site of the ribosome, but puromycin cannot bind to the P site, and translocation does not take place, blocking further elongation of the protein. Because tRNA structure is similar in all organisms, puromycin inhibits translation in both bacterial and eukaryotic cells; consequently, puromycin kills eukaryotic cells along with bacteria and is sometimes used in cancer therapy to destroy tumor cells.

Although these antibiotics act by blocking specific steps in translation, it is important to recognize that antibiotics may also have other effects that contribute to the killing of bacteria. Because different antibiotics block different steps in protein synthesis, antibiotics are frequently used to study protein synthesis.

Chapter 15 Review

CHAPTER SUMMARY

Section 15.1 Learning Objective: Be able to describe the relationship between genes and proteins.

- George Beadle and Edward Tatum developed the one gene, one enzyme hypothesis, which proposed that each gene specifies one enzyme; this hypothesis was later modified to become the one gene, one polypeptide hypothesis.
- Proteins are composed of 20 different amino acids. The amino acids in a protein are linked together by peptide bonds. The resulting polypeptide chains fold and associate to produce the secondary, tertiary, and quaternary structures of proteins.

Section 15.2 Learning Objective: Be able to explain how the genetic code was deciphered and give characteristics of the genetic code.

- Solving the genetic code required several different approaches, including the use of synthetic mRNAs with random sequences and short mRNAs that bind ribosomes and tRNAs.
- The genetic code is a triplet code: three nucleotides specify a single amino acid. It is also degenerate (meaning that more than

one codon may specify an amino acid), nonoverlapping, and universal (almost).

- Different tRNAs (isoaccepting tRNAs) may accept the same amino acid. Different codons may pair with the same anticodon through wobble, which can exist at the third position of the codon and allows some nonstandard pairing of bases in this position.
- The reading frame is set by the initiation codon. The end of the protein-coding sequence of an mRNA is marked by one of three termination codons.

Section 15.3 Learning Objective: Be able to describe the process by which amino acids are assembled into a protein through translation of an mRNA.

- Protein synthesis comprises four steps: (1) the binding of amino acids to the appropriate tRNAs, (2) initiation, (3) elongation, and (4) termination.
- The binding of an amino acid to the appropriate tRNA requires the presence of a specific aminoacyl-tRNA synthetase and ATP. The amino acid is attached by its carboxyl end to the 3′ end of the tRNA.
- In bacterial translation initiation, the small ribosomal subunit attaches to the mRNA and is positioned over the initiation codon. It is joined by the initiator tRNA and its associated amino acid (*N*-formylmethionine in bacterial cells) and later by the large ribosomal subunit. Initiation requires several initiation factors and GTP.

- In elongation, a charged tRNA enters the A site of a ribosome, a peptide bond is formed between the amino acids in the A and P sites, and the ribosome moves (translocates) along the mRNA to the next codon. Elongation requires several elongation factors as well as GTP.
- Translation is terminated when the ribosome encounters one of the three termination codons. Release factors and GTP are required to bring about termination.

Section 15.4 Learning Objective: Describe polyribosomes, mRNA surveillance, posttranslational changes to proteins, and the effect of antibiotics on translation.

- Each mRNA molecule may be simultaneously translated by several ribosomes, producing a structure called a polyribosome.
- Cells possess mRNA surveillance mechanisms that eliminate mRNAs with errors that may create problems in translation.
- Some proteins undergo posttranslational modification.
- Many antibiotics work by interfering with translation because many aspects of translation differ in bacteria and eukaryotes.

WORKED PROBLEMS

Problem 1

A series of auxotrophic mutants were isolated in *Neurospora*. Examination of fungi containing these mutations revealed that they grew on a minimal medium to which various compounds (A, B, C,

and D) were added; growth responses to each of the four compounds are presented in the following table. Give the order of compounds A, B, C, and D in a biochemical pathway. Outline a biochemical pathway that includes these four compounds, and indicate which step in the pathway is affected by each of the mutations.

	Compound			
Mutation number	**A**	**B**	**C**	**D**
134	+	+	−	+
276	+	+	+	+
987	−	−	−	+
773	+	+	+	+
772	−	−	−	+
146	+	+	−	+
333	+	+	−	+
123	−	+	−	+

» Solution Strategy

What information is required in your answer to the problem?

The order of compounds A–D in a biochemical pathway; for each mutation, which step in the pathway is blocked by the mutation.

What information is provided to solve the problem?

For each mutant, whether it grew on a minimal medium to which compounds A, B, C, or D were added.

For help with this problem, review:

The One Gene, One Enzyme Hypothesis in Section 15.1.

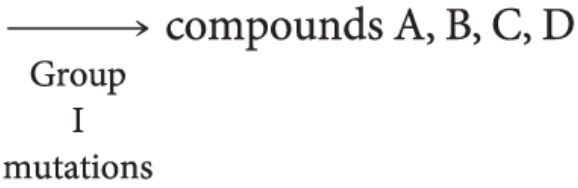
» Solution Steps

Mutation		Compound			
Group	Number	A	B	C	D
I	276	+	+	+	+
	773	+	+	+	+
II	134	+	+	−	+
	146	+	+	−	+
	333	+	+	−	+

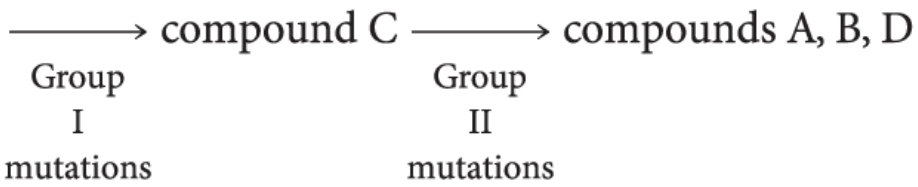
III	123	−	+	−	+
IV	987	−	−	−	+
	772	−	−	−	+

Hint: If a compound is added after the block, it will allow the mutant to grow; if a compound is added before the block, it will have no effect.

Mutants in group I will grow if compound A, B, C, or D is added to the medium, so these mutations must block a step before the production of all four compounds:

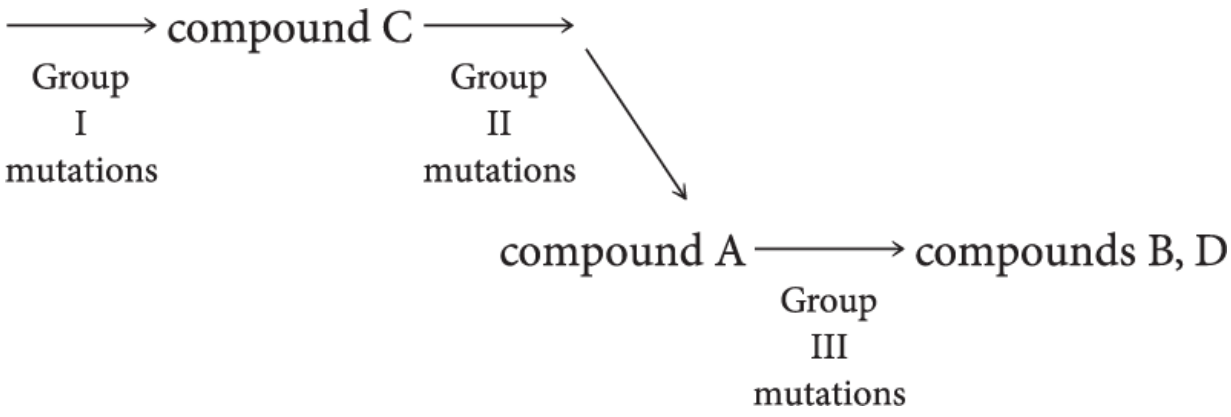


Group II mutants will grow if compound A, B, or D is added but not if compound C is added. Thus, compound C comes before A, B, and D, and group II mutations block the conversion of compound C into one of the other compounds:



Description
An arrow is pointing from left to right and text at the head of the arrow reads: compound C followed by another arrow from left to right and text at the head reads: Compounds A, B, D. The first arrow 1 is labeled group 1 mutations and second arrow is labeled group 2 mutations.

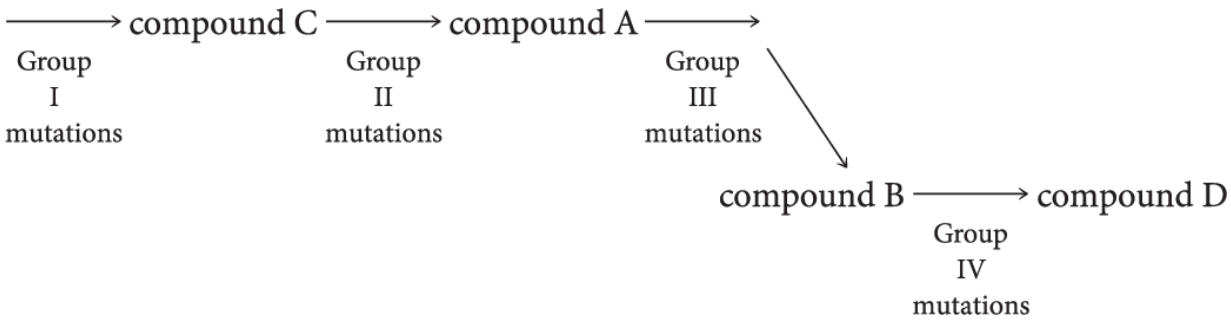
Group III mutants will grow if compound B or D is added but not if compound A or C is added. Thus, group III mutations block steps that follow the production of A and C; we have already determined that compound C precedes A in the pathway, so A must be the next compound in the pathway:



Description
Pointing left to right is an arrow labeled group 1 mutations with the text: compound C. To the right of compound C an arrow labeled group 2 mutations points to the tail of an arrow angled downward. The head of the downward arrow points to the text: compound A. An arrow pointing right from compound A is labeled group 3 mutations and points to the text: compounds B, D.

Finally, mutants in group IV will grow if compound D is added but not if compound A, B, or C is added. Thus, compound D is the fourth

compound in the pathway, and mutations in group IV block the conversion of B into D:



Description
Pointing left to right is an arrow labeled group 1 mutations that points to the text: compound C. To the right of compound C, an arrow labeled group 2 mutations is pointing to the text: compound A and an arrow to the right of compound A is pointing to the tail of an arrow angled downward. The head of the downward arrow points to the text: compound B. An arrow pointing right from compound B is labeled group 4 mutations and points to the text: compound D.

Problem 2

A template strand in bacterial DNA has the following base sequence:

$$5'\text{–AGGTTTAACGTGCAT–}3'$$

What amino acids are encoded by this sequence?

» Solution Strategy

What information is required in your answer to the problem?

The list of amino acids encoded by the given sequence.

What information is provided to solve the problem?

- The DNA sequence of the template strand.
- The 5′ and 3′ ends of the template sequence.
- The amino acids encoded by different codons (**Figure 15.10**).

For help with this problem, review:

The Degeneracy of the Code in Section 15.2.

» Solution Steps

To answer this question, we must first work out the mRNA sequence that will be transcribed from this DNA sequence:

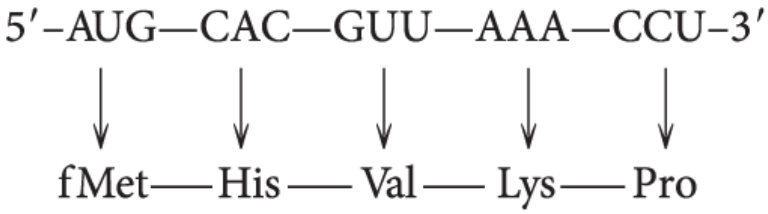
$$\begin{aligned}&\text{DNA template strand:} && 5'-\text{AGGTTTAACGTGCAT}-3'\\ &\text{mRNA copied from DNA:} && 3'-\text{UCCAAAUUGCACGUA}-5'\end{aligned}$$

An mRNA is translated 5′→3′, so it will be helpful if we turn the RNA molecule around with the 5′ end on the left:

$$\text{mRNA copied from DNA: } 5'-\text{AUGCACGUUAAACCU}-3'$$

Recall: The mRNA is antiparallel and complementary to the DNA template strand.

The codons consist of groups of three nucleotides that are read successively after the first AUG codon; using Figure 15.10, we can determine that the amino acids are



Description
Five codons from 5 prime to 3 prime ends of the R N A sequence, from left to right, along with their respective amino acids, are as follows:

A U G, f methionine; C A C, histidine; G U U, valine; A A A, lysine; and C C U, proline.

Problem 3

The following triplets constitute anticodons found on a series of tRNAs. Name the amino acid carried by each of these tRNAs.

a. $5'–UUU–3'$
b. $5'–GAC–3'$
c. $5'–UUG–3'$
d. $5'–CAG–3'$

» Solution Strategy

What information is required in your answer to the problem?

The amino acid carried by each tRNA.

What information is provided to solve the problem?

- The sequence on the anticodon of each tRNA.
- The amino acids encoded by different codons (**Figure 15.10**).

For help with this problem, review:

The Degeneracy of the Code in Section 15.2.

» Solution Steps

Recall: Codons are antiparallel and complementary to the anticodons.

To solve this problem, we first determine the codons with which these anticodons pair and then look up the amino acid specified by each codon in **Figure 15.10**. For part *a*, the anticodon is $5'$–UUU–$3'$. According to the wobble rules, U in the first position of the anticodon can pair with either A or G in the third position of the codon, so there are two codons that can pair with this anticodon:

$$\begin{aligned} &\text{Anticodon}: && 5'-\text{UUU}-3' \\ &\text{Codon}: && 3'-\text{AAA}-5' \\ &\text{Codon}: && 3'-\text{GAA}-5' \end{aligned}$$

Hint: Consult the wobble rules in Table 15.2.

Listing these codons in the conventional manner, with the $5'$ end on the left, we have

$$\begin{aligned} \text{Codon}: &\quad 5'-\text{AAA}-3' \\ \text{Codon}: &\quad 5'-\text{AAG}-3' \end{aligned}$$

According to **Figure 15.10**, both codons specify the amino acid lysine (Lys). Recall that the wobble in the third position allows more than one codon to specify the same amino acid, so any wobble that exists should produce the same amino acid as the standard base pairings would, and we do not need to figure the wobble to answer this question. The answers for parts *b*, *c*, and *d* are

b. $\begin{aligned} \text{Anticodon}: &\quad 5'-\text{GAC}-3' \\ \text{Anticodon}: &\quad 3'-\text{CAG}-5' \\ \text{Codon}: &\quad 5'-\text{GUC}-3' \text{ encodes Val} \end{aligned}$

c. $\begin{aligned} \text{Anticodon}: &\quad 5'-\text{UUG}-3' \\ \text{Anticodon}: &\quad 3'-\text{GUU}-5' \\ \text{Codon}: &\quad 5'-\text{CAA}-3' \text{ encodes Gln} \end{aligned}$

d. $\begin{aligned} \text{Anticodon}: &\quad 5'-\text{CAG}-3' \\ \text{Anticodon}: &\quad 3'-\text{GUC}-5' \\ \text{Codon}: &\quad 5'-\text{CUG}-3' \text{ encodes Leu} \end{aligned}$

IMPORTANT TERMS

one gene, one enzyme hypothesis
one gene, one polypeptide hypothesis
amino acid
peptide bond
polypeptide
sense codon
degenerate genetic code
synonymous codon
isoaccepting tRNA
wobble
nonoverlapping genetic code
reading frame
initiation (start) codon
stop (termination or nonsense) codon
universal genetic code
aminoacyl-tRNA synthetase
tRNA charging
initiation factors (IF-1, IF-2, IF-3)
30S initiation complex
70S initiation complex
cap-binding complex (CBC)
aminoacyl (A) site
peptidyl (P) site
exit (E) site
elongation factor Tu (EF-Tu)
elongation factor Ts (EF-Ts)
translocation
elongation factor G (EF-G)
release factors (RF-1, RF-2, RF-3)

polyribosome
mRNA surveillance
nonsense-mediated mRNA decay (NMD)
transfer–messenger RNA (tmRNA)
nonstop mRNA decay
no-go decay (NGD)
molecular chaperone
signal sequence

ANSWERS TO CONCEPT CHECKS

1. B → A → C
2. The amino acid sequence (primary structure) of the protein
3. b
4. d
5. The initiation codon in bacteria encodes *N*-formylmethionine; in eukaryotes, it encodes methionine. Termination codons do not specify amino acids.
6. c
7. The Shine–Dalgarno sequence
8. a
9. b

Chapter 15 Assessment

COMPREHENSION QUESTIONS

Section 15.1

1. What is the one gene, one enzyme hypothesis? Why was this hypothesis an important advance in our understanding of genetics?

Section 15.2

2. What different methods were used to help break the genetic code? What did each method reveal, and what were the advantages and disadvantages of each one?

3. What are isoaccepting tRNAs?

4. What is the significance of the fact that many synonymous codons differ only in the third nucleotide position?

5. Define the following terms as they apply to the genetic code:

   a. Reading frame

   b. Overlapping code

   c. Nonoverlapping code

   d. Initiation codon

   e. Termination codon

   f. Sense codon

   g. Nonsense codon

   h. Universal code

   i. Nonuniversal codons

6. How is the reading frame of a nucleotide sequence set?

Section 15.3

7. How are tRNAs linked to their corresponding amino acids?
8. What role do the initiation factors play in protein synthesis?
9. How does the process of initiation differ in bacterial and eukaryotic cells?
10. Give the elongation factors used in bacterial translation, and explain the role played by each factor in translation.
11. What events bring about the termination of translation?
12. Compare and contrast the process of protein synthesis in bacterial and eukaryotic cells, giving similarities and differences in the process of translation in these two types of cells.

Section 15.4

13. How do prokaryotic cells overcome the problem of a stalled ribosome on an mRNA that has no termination codon? How do eukaryotic cells solve this problem?
14. What are some types of posttranslational modification of proteins?
15. Explain how some antibiotics work by affecting the process of protein synthesis.

APPLICATION QUESTIONS AND PROBLEMS

Section 15.1

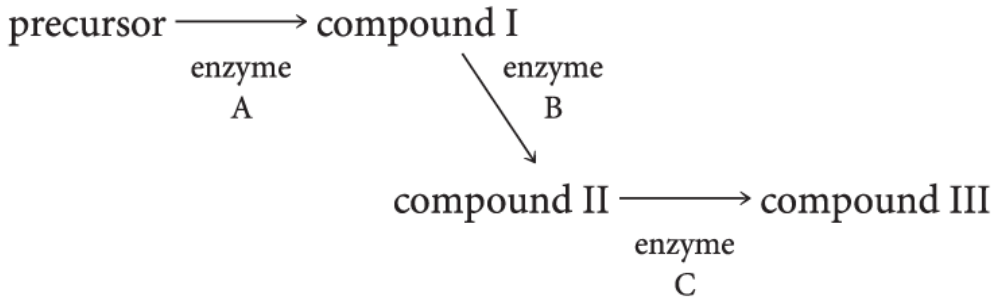
16. *Sydney Brenner isolated *Salmonella typhimurium* mutants that were implicated in the biosynthesis of tryptophan and would not grow on a minimal medium. When these bacterial mutants were tested on a minimal medium to which one of four compounds (indole glycerol phosphate, indole, anthranilic acid, and tryptophan) had been added, the growth responses shown in the following table were obtained.

Mutant	Minimal medium	Anthranilic acid	Indole glycerol phosphate	Indole	Tryptophan
trp-1	–	–	–	–	+
trp-2	–	–	+	+	+
trp-3	–	–	–	+	+

trp-4	–	–	+	+	+
trp-6	–	–	–	–	+
trp-7	–	–	–	–	+
trp-8	–	+	+	+	+
trp-9	–	–	–	–	+
trp-10	–	–	–	–	+
trp-11	–	–	–	–	+

Give the order of indole glycerol phosphate, indole, anthranilic acid, and tryptophan in a biochemical pathway leading to the synthesis of tryptophan. Indicate which step in the pathway is affected by each of the mutations.

17. Compounds I, II, and III are in the following biochemical pathway:



Description
Pointing left to right the precursor is followed by an arrow labeled enzyme A, pointing to compound 1. An arrow angled downward from compound 1 is labeled enzyme B and points to compound 2. An arrow pointing right from compound 2 is labeled enzyme C and points to compound 3.

Mutation *a* inactivates enzyme A, mutation *b* inactivates enzyme B, and mutation *c* inactivates enzyme C. Mutants, each having one of these defects, were tested on a minimal medium to which compound I, II, or III was added. Fill in the results expected of these tests by placing a plus sign (+) for growth or a minus sign (–) for no growth in the following table.

	Minimal medium to which is added		
Strain with mutation	**Compound I**	**Compound II**	**Compound III**

a	
b	
c	

Section 15.2

18. A geneticist conducts the experiment outlined in **Figure 15.8**, but this time she combines guanine nucleotides (instead of uracil) with polynucleotide phosphorylase. Radioactively labeled protein should appear in which tube?

19. For the experiment outlined in **Figure 15.8**, could Nirenberg and Matthaei have substituted RNA polymerase instead of polynucleotide phosphorylase without otherwise modifying the experiment? Why or why not?

20. *Assume that the number of different types of bases in RNA is four. What would be the minimum codon size (number of nucleotides) required to specify all amino acids if the number of different types of amino acids in proteins were (a) 2, (b) 8, (c) 17, (d) 45, (e) 75?

21. *How many codons would be possible in a triplet code if only three bases (A, C, and U) were used?

22. *Referring to the genetic code presented in **Figure 15.10**, give the amino acids specified by the following bacterial mRNA sequences.

    a. $5'$–AUGUUUAAAUUUAAAUUUUGA–$3'$

    b. $5'$–AGGGAAAUCAGAUGUAUAUAUAUAUAUGA–$3'$

    c. $5'$–UUUGGAUUGAGUGAAACGAUGGAUGAAAGAUUUCUCGCUUGA–$3'$

    d. $5'$–GUACUAAGGAGGUUGUAUGGGUUAGGGGACAUCAUUUUGA–$3'$

23. A nontemplate strand of bacterial DNA has the following base sequence. What amino acid sequence will be encoded by this sequence?

$$5'-\text{ATGATACTAAGGCCC}-3'$$

24. The following amino acid sequence is found in a tripeptide: Met-Trp-His. Give all possible nucleotide sequences on the mRNA, on the template strand of DNA, and on the nontemplate strand of DNA that can encode this tripeptide.

25. How many different mRNA sequences can encode a polypeptide chain with the amino acid sequence Met-Leu-Arg? (Be sure to include the stop codon.)

26. *A series of tRNAs have the following anticodons. Consider the wobble rules listed in **Table 15.2**, and give all possible codons with which each tRNA can pair.

    a. 5′–GGC–3′

    b. 5′–AAG–3′

    c. 5′–IAA–3′

    d. 5′–UGG–3′

    e. 5′–CAG–3′

27. A researcher creates random copolymers of three nucleotides each by mixing polynucleotide phosphorylase with guanine and adenine nucleotides in a ratio of 5 guanine nucleotides to 1 adenine. Give the different copolymers produced and their theoretical proportions.

28. Assume that the nucleotide at the 5′ end of the first tRNA's anticodon (the tRNA on the left) in **Figure 15.11** was mutated from G to U. Give all codons with which the new, mutated anticodon could pair.

29. Which of the following amino acid changes could result from a mutation that changed a single base? For each change that could result from the alteration of a single base, determine which position of the codon (first, second, or third nucleotide) in the mRNA must be altered for the change to result.

    a. Leu → Gln

    b. Phe → Ser

    c. Phe → Ile

    d. Pro → Ala

    e. Asn → Lys

    f. Ile → Asn

Section 15.3

30. *Arrange the following components of translation in the approximate order in which they would appear or be used in prokaryotic protein synthesis:

    70S initiation complex

30S initiation complex

release factor 1

elongation factor G

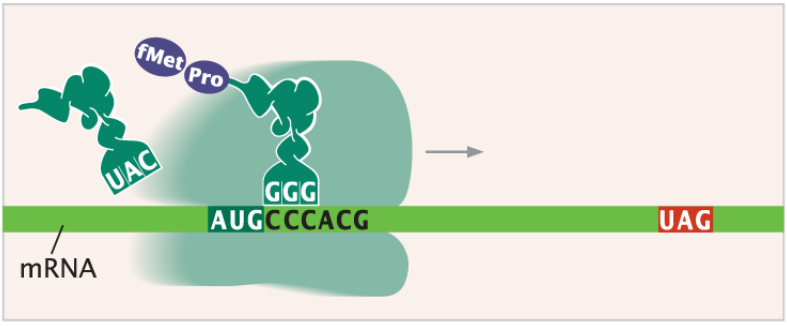
initiation factor 3

elongation factor Tu

fMet-tRNA$_i^{fMet}$

31. Examine the tRNA in **Figure 15.14**. What do you think would be the potential effect of a mutation in the part of the tRNA gene that encodes (a) the acceptor stem; (b) the anticodon; (c) one of the red nucleotide positions?

32. The following diagram illustrates a step in the process of translation. Identify the following elements on the diagram.



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Description
The diagram starts with an m R N A strand with a ribosome and a t R N A attached.

A t R N A without an amino acid with U A C at its base, is on the left side of the ribosome. A t R N A with anticodon G G G sits on the m R N A at sequence C C C with amino acids f met and proline attached to it. The codon on the left of R N A sequence C C C is A U G and the codon on the right is A C G. An arrow is drawn pointing right from the ribosome. A red codon labeled U A G is present on the right end of the m R N A strand.

a. 5′ and 3′ ends of the mRNA

b. A, P, and E sites

c. Start codon

d. Stop codon

e. Amino and carboxyl ends of the newly synthesized polypeptide chain

f. Approximate location of the next peptide bond that will be formed

g. Place on the ribosome where release factor 1 will bind

33. *Refer to the diagram in Problem 32 to answer the following questions.

a. What will be the anticodon of the next tRNA added to the A site of the ribosome?

b. What will be the next amino acid added to the growing polypeptide chain?

34. *A synthetic mRNA added to a cell-free protein-synthesizing system produces a peptide with the following amino acid sequence: Met-Pro-Ile-Ser-Ala. What would be the effect on translation if the following components were omitted from the cell-free protein-synthesizing system? What, if any, type of protein would be produced? Explain your reasoning.

a. Initiation factor 3

b. Initiation factor 2

c. Elongation factor Tu

d. Elongation factor G

e. Release factors RF-1, RF-2, and RF-3

f. ATP

g. GTP

35. CONNECT For each of the following sequences, place a check mark in the appropriate space to indicate the process *most immediately* affected by deleting the sequence. Choose only one process for each sequence (i.e., one check mark per sequence). For help, review Chapters 12 to 15.

	Process most immediately affected by deletion			
Sequence deleted	**Replication**	**Transcription**	**RNA processing**	**Translation**
a. *ori* site				
b. 3′ splice site consensus				
c. Poly(A) tail				
d. Terminator				
e. Start codon				
f. −10 consensus				
g. Shine–Dalgarno				

36. DATA ANALYSIS MicroRNAs are small RNA molecules that bind to the 3′ end of mRNA and suppress translation (see Section 14.5). Some eukaryotic mRNAs have internal ribosome-binding sites downstream of the 5′ cap, where ribosomes normally bind. In one investigation, miRNAs did not suppress translation by ribosomes that had attached to internal ribosome-binding sites (R. S. Pillai et al. 2005. *Science* 309:1573–1576). What does this finding suggest about how miRNAs suppress translation?

37. Give the amino acid sequence of the protein encoded by the mRNA in **Figure 15.21**.

Section 15.4

38. CONNECT (a) Label the following features on the following diagram. Review Chapter 13 for components associated with transcription. (b) Is this figure from a bacterial or a eukaryotic cell? Explain your reasoning.

    a. DNA

    b. mRNA

    c. 5′ and 3′ ends of the DNA

    d. 5′ and 3′ ends of the mRNA

    e. ribosomes

    f. approximate location of promoter

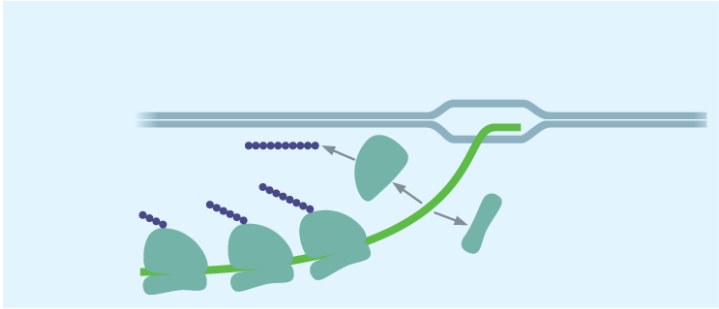
    g. approximate location of terminator

    h. direction of transcription

    i. start codon

    j. Shine–Dalgarno sequence

    k. stop codon



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Description
On the left, three ribosomes with growing polypeptide chains are attached to the m R N A strand. The m R N A sequence separates large and small subunits of ribosome located above and below the strand respectively, as it approaches the bubble in D N A strand. This is followed by dissociation of polypeptide chain towards the right end.

39. *Mutations that introduce stop codons cause a number of genetic diseases. For example, from 2% to 5% of the people who have cystic fibrosis possess a mutation that causes a premature stop codon in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). This premature stop codon produces a truncated form of CFTR that is nonfunctional and results in the symptoms of cystic fibrosis (see Section 5.1). One possible way to treat people with genetic diseases caused by these types of mutations is to trick the ribosome into reading through the stop codon, inserting an amino acid in its place. Although the protein produced may have one altered amino acid, it is more likely to be at least partly functional than is the truncated protein produced when the ribosome stalls at the stop codon. Indeed, geneticists have conducted clinical trials of a drug called PTC124 on people with cystic fibrosis. This drug interferes with the ribosome's ability to correctly read stop codons (C. Ainsworth. 2005. *Nature* 438:726–728). On the basis of what you know about the mechanism of nonsense-mediated mRNA decay (NMD), would you expect NMD to pose a problem for this type of treatment? Why or why not?

SCIENCE PHOTO LIBRARY/Getty Images.

Patient with cystic fibrosis undergoing respiratory therapy.

CHALLENGE QUESTIONS

Section 15.2

40. The redundancy of the genetic code means that some amino acids are specified by more than one codon. For example, the amino acid leucine is specified by six different codons. Within a genome, synonymous codons are not present in equal numbers; some synonymous codons appear much more frequently than others, and the preferred codons differ among species. For example, in one species, the codon UUA might be used most often to specify leucine, whereas in another species, the codon CUU might be used most often. Speculate on a reason for this bias in codon us and why the preferred codons are not the same in all organisms.

Section 15.3

41. CONNECT In what ways are spliceosomes (see Chapter 14) and ribosomes similar? In what ways are they different? Can you suggest some possible reasons for their similarities?

42. *Several experiments were conducted to obtain information about how the eukaryotic ribosome recognizes the AUG start codon. In one experiment, the gene that encodes methionine initiator tRNA ($tRNA_i^{Met}$) was located and changed; specifically, the nucleotides that specify the anticodon on $tRNA_i^{Met}$ were mutated so that the anticodon in the tRNA was 5′–CCA–3′ instead of 5′–CAU–3′. When this mutated gene was placed in a eukaryotic cell, protein synthesis took place, but the proteins produced were abnormal. Some of these proteins contained extra amino acids, and others contained fewer amino acids than normal.

a. What do these results indicate about how the ribosome recognizes the starting point for translation in eukaryotic cells? Explain your reasoning.

b. If the same experiment had been conducted on bacterial cells, what results would you expect?

c. Explain why some of the proteins produced contained extra amino acids while others contained fewer amino acids than normal.

43. CONNECT In bacteria, the process of translation affects the speed of transcription (see Chapter 13)—efficient binding and progression of ribosomes along the mRNA increase the speed of transcription, whereas the absence of ribosomes on the mRNA slows the movement of RNA polymerase along the DNA. Treatment with an antibiotic that inhibits translation slows the progression of RNA polymerase along the DNA. Likewise, if ribosomal proteins are mutated in a way that slows or inhibits translation, the process of transcription also slows. Furthermore, mutations in ribosomes that speed up the process of translation also accelerate transcription. Propose a mechanism for how the process of translation could affect the speed of transcription.

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 15.1

1. Archibald Garrod was an English physician who first proposed that genes encode enzymes. Like the work of Gregor Mendel (see Chapter 3), his discovery had little impact on his contemporaries and was not widely accepted until many years later. Why are important discoveries in science sometimes not accepted immediately? Why does it often take years before they are generally accepted by other scientists?

2. Based on what you have now learned about how genes code for proteins, do you think the one gene, one polypeptide hypothesis is correct today? Why or why not?

3. Compare and contrast the structure and function of proteins and nucleic acids. How are they similar, and how are they different? How do their different structures contribute to their different functions?

Section 15.2

4. A triplet code with three nucleotides per codon is the most efficient way to encode the 20 different amino acids. Why would cells be expected to use the most efficient code? In other

words, what might be the advantages of using an efficient code?

5. Some evidence suggests that synonymous codons are not really synonymous. Sometimes mutations that change a codon to a synonymous codon produce an effect on the phenotype, even though the amino acid sequence of the protein is the same. Propose some ways in which synonymous codons might have different phenotypic effects.

6. There are three termination codons (UAA, UAG, and UGA) but usually only one initiation codon (AUG) is used. Propose some possible reasons for why there are more termination codons than initiation codons.

7. Exceptions to the universality of the genetic code were once thought to be rare, but more and more exceptions are being found. As mentioned in the text, one study found that a significant proportion of bacteria in the environment use nonuniversal codons. Can you think of any advantages for an organism using nonuniversal codons?

Section 15.3

8. Structural analysis of bacterial release factor 1 (RF-1) and release factor 2 (RF-2) reveals that these proteins are similar in size and shape to a tRNA molecule. This similarity has sometimes been called molecular mimicry. Why might RF-1 and RF-2 have evolved to mimic tRNAs?

Section 15.4

9. In humans, there may be three times as many proteins as genes. If each gene encodes a protein, how can there be more proteins than genes?

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Create a concept map that outlines the genetic cause of isolated congenital asplenia (ICA). Include at least the following terms in your concept map: spleen, ICA, immunity, infections, *RSPA* gene, RSPA protein, ribosome, protein synthesis, ribosomopathy.

Section 15.1

2. Draw a concept map that summarizes the relationship between genes and proteins, incorporating the following terms into your map: gene; protein, one gene, one enzyme hypothesis, one gene, one polypeptide hypothesis, amino acids, peptide bond, primary structure, secondary structure, tertiary structure, quaternary structure, domain.

Section 15.2

3. Create a concept map that outlines the characteristics of the genetic code.

Section 15.3

4. Draw a concept map that reviews the major components involved in the four steps of bacterial translation and their functions.

Section 15.4

5. Summarize mRNA surveillance mechanisms with a concept map that includes the following terms: translation, mRNA, mRNA surveillance, nonsense mutations, nonsense-mediated mRNA decay, stalled ribosome, transfer-messenger RNA, alternative ribosome-rescue factors, nonstop decay, no-go decay.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 16

Control of Gene Expression in Bacteria

Pasieka/Science Source.

The expression of genes in bacteria is often regulated through operons, groups of genes that are transcribed as units. Shown here is *Escherichia coli*, a common bacterium found in the intestinal tracts of mammals.

Operons and the Noisy Cell

In 2011, geneticists from around the world celebrated the 50th anniversary of the operon. An operon is a group of genes that share a common promoter and are transcribed as a unit, producing a single mRNA molecule that encodes several proteins. Typically, these proteins interact in some way; for example, the *trp* operon in the bacterium *Escherichia coli* encodes components of three enzymes that work together to synthesize the amino acid tryptophan. Operons control the expression of genes such as these by regulating their transcription. Genes in bacteria and archaea are often organized into operons, but operons are much less common in eukaryotes.

The operon was discovered through the elegant research of François Jacob and Jacques Monod, who worked at opposite ends of the attic floor of the Pasteur Institute in Paris. Jacob was studying bacteriophage λ, a virus that infects *E. coli*; Monod was analyzing the properties of β-galactosidase, an enzyme *E. coli* uses to metabolize the sugar lactose. One summer evening in 1958, Jacob had a flash of inspiration—he saw a connection between the research taking place at the two ends of the attic. Jacob recognized that the genes that induce phage reproduction were controlled in the same way as the genes that control production of β-galactosidase in *E. coli*. This realization led to a successful collaboration between Jacob and Monod; together, they eventually uncovered the structure and function of the *lac* operon. In 1965,

they were awarded the Nobel Prize in physiology or medicine, along with their collaborator, André Lwoff.

Following Jacob and Monod's discovery of the *lac* operon, other operons were discovered in bacteria, and a great deal of research focused on the mechanism of operon function. Despite extensive research on *how* operons work, much less was known about *why* operons exist: Why do prokaryotes have them while most eukaryotes don't? Why are some genes included in operons while others are not? These questions intrigued Oleg Igoshin, at Rice University, and Christian Ray, at the University of Texas MD Anderson Cancer Center. Igoshin and Ray are computational biologists, a new breed of scientists who use complex mathematics to study fundamental problems of biology. They took what might seem like an unlikely approach to the question of why operons exist. Instead of growing bacteria, inducing mutations, and examining DNA, they developed a series of mathematical models of gene networks that could be run on a computer. Using these models, they looked at how genes functioned when grouped into operons and when regulated separately.

Igoshin and Ray knew that random fluctuations in the levels of transcription and translation occur naturally. Because of these fluctuations—noise in the system—the amounts of different proteins can vary widely; thus, the amount of a protein produced might be more or less than is optimal for cell growth and survival.

Igoshin and Ray hypothesized that coordinating the transcription of several genes through an operon structure might reduce noise in the system and permit more finely tuned control over gene expression.

To test their hypothesis, Igoshin and Ray ran computer models for six different types of interactions between the products of the types of genes that are potentially found in operons. Their models showed that for some types of protein interactions, grouping genes together in an operon decreases the biochemical noise. For other types of protein interactions, grouping the genes in operons actually increases the noise. Thus, the operon structure has the potential to increase or decrease noise depending on which genes are grouped together.

Igoshin and Ray then examined genes that are actually found in operons in *E. coli*, and they discovered that operons containing genes whose interactions decrease noise were more common than expected on a random basis. Conversely, operons whose gene interactions increase noise were less common than expected. They concluded that operons have evolved as a way for the cell to couple transcription of multiple genes so as to reduce biochemical noise in the cell, allowing the cell to more finely tune the relative proportions of the proteins encoded by the operon. Igoshin and Ray speculated that operons are less common in eukaryotes because the larger volume of eukaryotic cells reduces the effect of random

fluctuations and, perhaps, because eukaryotes have other mechanisms (such as changes in chromatin structure) to couple the transcription of multiple genes.

THINK-PAIR-SHARE

- François Jacob and Jacques Monod began their successful collaboration, in part, because they worked on the same floor of the Pasteur Institute. In today's technology-filled world, where people can easily communicate by email and videoconferencing, is physical proximity still of any value in collaborating in research? Why or why not?
- What other mechanisms, in addition to operons, might allow groups of genes to be regulated together?

This chapter is the first of two chapters about **gene regulation**, the mechanisms and systems that control the expression of genes. In this chapter, we consider systems of gene regulation in bacteria. We begin by considering the necessity for gene regulation, the levels at which gene expression is controlled, and the difference between genes and regulatory elements. We then examine the structure and function of operons, and we consider gene regulation in some specific examples of operons, including the *lac* operon studied by Jacob and Monod. Finally, we discuss several types of bacterial gene regulation that are facilitated by RNA molecules. In Chapter 17, we will discuss mechanisms of gene regulation in eukaryotic genomes.

THINK-PAIR-SHARE Question 1

16.1 The Regulation of Gene Expression Is Critical for All Organisms

A major theme of molecular genetics is the central dogma, which states that genetic information flows from DNA to RNA to proteins (see Figure 10.17a). Although the central dogma provided a molecular basis for the connection between genotype and phenotype, it failed to address a critical question: How is the flow of information along this molecular pathway *regulated*?

Consider *E. coli*, a bacterium that resides in your large intestine. Your eating habits completely determine the nutrients available to this bacterium: it can neither seek out nourishment when nutrients are scarce nor move away when confronted with an unfavorable environment. The bacterium makes up for its inability to alter the external environment by being internally flexible. For example, if glucose is present, *E. coli* uses it to generate ATP; if there's no glucose, it uses lactose, arabinose, maltose, xylose, or any of a number of other sugars. When amino acids are available, *E. coli* uses them to synthesize proteins; if a particular amino acid is absent, *E. coli* produces the enzymes needed to synthesize that amino acid. Thus, *E. coli* responds to environmental changes by rapidly altering its biochemistry. This biochemical flexibility, however, has a high price. Constantly producing all the enzymes necessary for every environmental condition would be energetically expensive. So how does *E. coli* maintain biochemical flexibility while optimizing energy efficiency?

The answer is through gene regulation. Bacteria carry the genetic information for synthesizing many proteins, but only a subset of that genetic information is expressed at any time. When the environment changes, new genes are expressed, and proteins appropriate for the new environment are

synthesized. For example, if a carbon source appears in the environment, genes encoding enzymes that take up and metabolize that carbon source are quickly transcribed and translated. When that carbon source disappears, the genes that encode those enzymes are shut off.

Multicellular eukaryotic organisms face a further challenge. Individual cells in a multicellular organism are specialized for particular tasks. The proteins produced by a nerve cell, for example, are quite different from those produced by a white blood cell. But although they differ in shape and function, a nerve cell and a white blood cell still carry the same genetic instructions.

A multicellular organism's challenge is to bring about the differentiation of cells that have a common set of genetic instructions (the process of development). This challenge is met through gene regulation: all of an organism's cells carry the same genetic information, but only a subset of genes are expressed in each cell type; genes needed for other cell types are not expressed. Gene regulation is therefore the key to both unicellular flexibility and multicellular specialization, and it is critical to the success of all living organisms.

The mechanisms of gene regulation were first investigated in bacterial cells, in which the availability of mutants and the ease of laboratory manipulation made it possible to unravel these mechanisms. When the study of these mechanisms in eukaryotic cells began, eukaryotic gene regulation seemed very different from bacterial gene regulation. However, as more and more information has accumulated about gene regulation, a number of common themes have emerged. Today, many aspects of gene regulation in bacterial and eukaryotic cells are recognized to be similar. Before examining specific elements of bacterial gene regulation (in this chapter) and eukaryotic gene

regulation (in Chapter 17), we briefly consider some themes of gene regulation common to all organisms.

CONCEPTS

In bacteria, gene regulation maintains internal flexibility, turning genes on and off in response to environmental changes. In multicellular eukaryotic organisms, gene regulation also brings about cell differentiation.

Genes and Regulatory Elements

In considering gene regulation in both bacteria and eukaryotes, we must distinguish between the DNA sequences that are transcribed and the DNA sequences that regulate the expression of other sequences. **Structural genes** encode proteins that are used in metabolism or biosynthesis or that play a structural role in the cell. On the other hand, the products of **regulatory genes**—either RNA or proteins—interact with other DNA sequences and affect the transcription or translation of those sequences. In many cases, the products of regulatory genes are DNA-binding proteins or RNA molecules that affect gene expression.

Bacteria and eukaryotes use regulatory genes to control the expression of many of their structural genes. However, a few structural genes, particularly those that encode essential cellular functions (often called housekeeping genes), are expressed continually and are therefore said to be **constitutive**. Constitutive genes are not regulated.

We will also encounter DNA sequences that are not transcribed at all but still play a role in regulating genes and other DNA sequences. These **regulatory**

elements affect the expression of DNA sequences to which they are physically linked. Regulatory elements are common in both bacterial and eukaryotic cells, and much of gene regulation in both types of organisms takes place through the action of proteins produced by regulatory genes that recognize and bind to regulatory elements.

The regulation of gene expression can occur through processes that stimulate gene expression, termed *positive control*, or through processes that inhibit gene expression, termed *negative control*. Bacteria and eukaryotes use both positive and negative control mechanisms to regulate their genes.

THINK-PAIR-SHARE Question 2

CONCEPTS

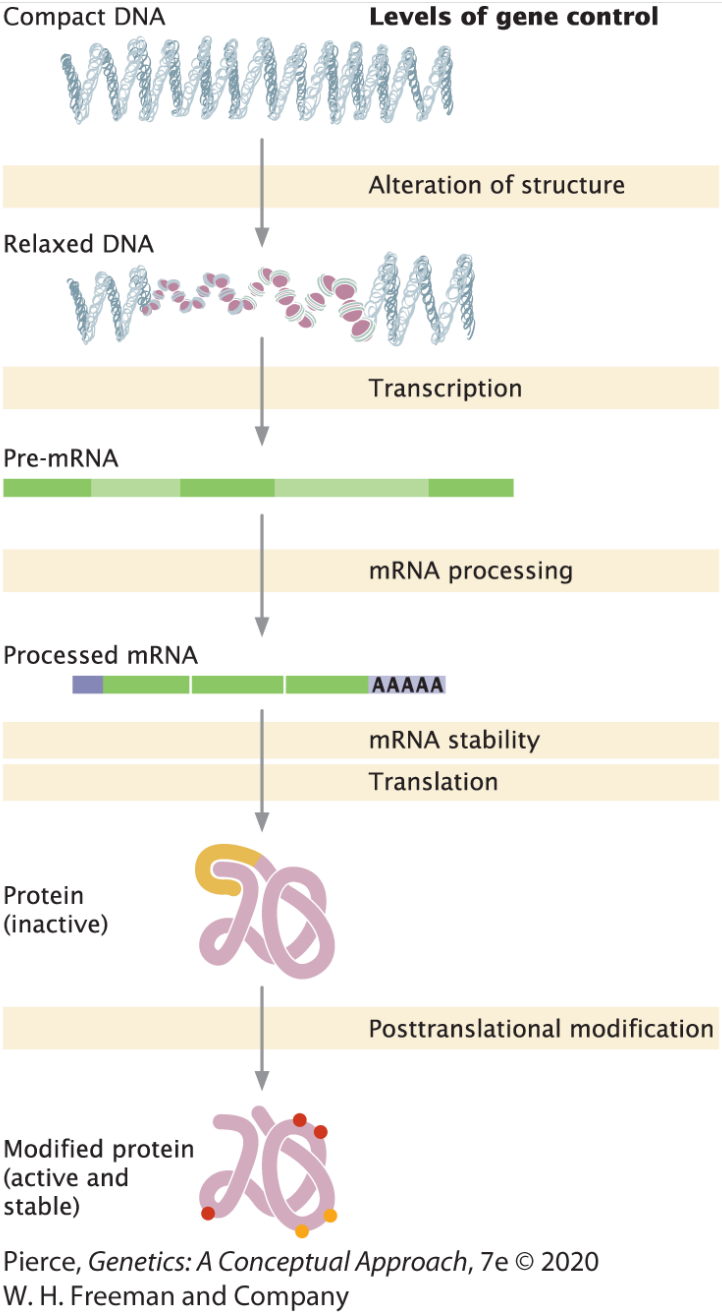
Structural genes encode proteins, while regulatory elements are DNA sequences that are not transcribed but affect the expression of genes. Positive control mechanisms stimulate gene expression, whereas negative control mechanisms inhibit gene expression.

✔ CONCEPT CHECK 1

What is a constitutive gene?

Levels of Gene Regulation

In both bacteria and eukaryotes, genes can be regulated at a number of levels along the pathway of information flow from genotype to phenotype (**Figure 16.1**).



16.1 Gene expression can be controlled at multiple levels.

Description

Text on the top reads, Levels of gene control. The first illustration is labeled, Compact D N A, and shows a horizontal ribbon of D N AText. Alteration of structure occurs and results in relaxed D N A. The illustration shows the central part of a D N A coil break apart into looser molecules. The D N A on the either side is still compact. Transcription occurs and a linear pre-m R N A is formed. m R N A processing occurs and processed m R N A is shown with poly A tail on its right end. m R N A stabilizes

and translation occurs. A coiled structure with a yellow part is shown, labeled protein (inactive). Posttranslational modification occurs and a modified protein (active and stable) is obtained. This protein has same coiled structure as inactive protein but has small red and yellow circles overlapping it.

First, genes can be regulated through the alteration of DNA or chromatin structure; this type of gene regulation takes place primarily in eukaryotes. Modifications to DNA or its packaging can help determine which sequences are available for transcription or the rate at which sequences are transcribed. DNA methylation and changes in chromatin structure are two processes that play a pivotal role in gene regulation.

A second point at which a gene can be regulated is at the level of transcription. For the sake of cellular economy, limiting the production of a protein early in the process makes sense, and transcription is an important point of gene regulation in both bacterial and eukaryotic cells.

A third potential point of gene regulation is mRNA processing. Eukaryotic mRNA is extensively modified before it is translated: a $5'$ cap is added, the $3'$ end is cleaved and polyadenylated, and introns are removed (see Chapter 14). These modifications determine the movement of the mRNA into the cytoplasm, whether the mRNA can be translated, the rate of translation, and the amino acid sequence of the protein produced, as well as mRNA stability. There is growing evidence that a number of regulatory mechanisms in eukaryotic cells operate at the level of mRNA processing. In prokaryotes, in which these types of mRNA processing are largely absent, control of gene expression occurs primarily at other points.

A fourth point for the control of gene expression is the regulation of mRNA stability. The amount of protein produced depends not only on the amount of mRNA synthesized but also on the rate at which the mRNA is degraded.

A fifth point of gene regulation is at the level of translation, a complex process requiring a large number of enzymes, protein factors, and RNA molecules (see Chapter 15). All of these factors, as well as the availability of amino acids, affect the rate at which proteins are produced and therefore provide points at which gene expression can be controlled. Translation can also be affected by sequences in mRNA, such as those in the 5′ and 3′ untranslated regions.

Finally, many proteins are modified after translation (see Chapter 15), and these modifications affect protein stability and whether the proteins become active; therefore, genes can be regulated through processes that affect posttranslational modification. Gene expression can be affected by regulatory activities at any or all of these points.

CONCEPTS

Gene expression can be controlled at any of a number of levels along the molecular pathway from DNA to protein, including DNA or chromatin structure, transcription, mRNA processing, mRNA stability, translation, and posttranslational modification.

✔ CONCEPT CHECK 2

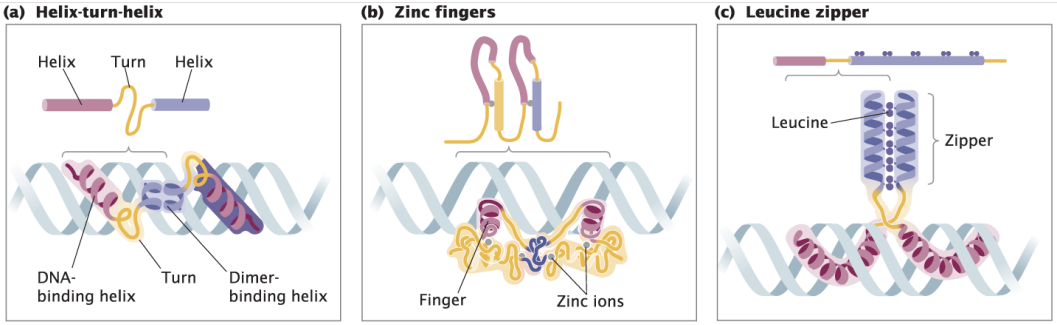
Why is transcription a particularly important level of gene regulation in both bacteria and eukaryotes?

DNA-Binding Proteins

Much of gene regulation in bacteria and eukaryotes is accomplished by proteins that bind to DNA sequences and affect their expression. These regulatory proteins generally have discrete functional parts—called **domains**,

typically consisting of 60 to 90 amino acids—that are responsible for binding to DNA. Within a domain, only a few amino acids actually make contact with the DNA. These amino acids (most commonly asparagine, glutamine, glycine, lysine, and arginine) often form hydrogen bonds with the bases or interact with the sugar–phosphate backbone of the DNA. Many DNA-binding proteins have additional domains that can bind other molecules, such as other regulatory proteins. By physically attaching to DNA, these proteins can affect the expression of a gene. Most DNA-binding proteins bind dynamically, which means that they transiently bind and unbind DNA and other regulatory proteins. Thus, although they may spend most of their time bound to DNA, they are never permanently attached. This dynamic nature means that other molecules can compete with DNA-binding proteins for regulatory sites on the DNA.

DNA-binding proteins can be grouped into several distinct types on the basis of characteristic structures, called *motifs*, found within the binding domain. Motifs are simple structures, such as alpha helices, that can fit into the major groove of the DNA double helix. For example, the helix-turn-helix motif (**Figure 16.2a**), consisting of two alpha helices connected by a turn, is common in bacterial regulatory proteins. The zinc-finger motif (**Figure 16.2b**), shared by many eukaryotic regulatory proteins, consists of a loop of amino acids containing a zinc ion. The leucine zipper (**Figure 16.2c**) is another motif found in a variety of eukaryotic DNA-binding proteins. These common DNA-binding motifs and others are summarized in **Table 16.1**.



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16.2 DNA-binding proteins can be grouped into several types on the basis of characteristic structures, or motifs.

Description

Part a is labeled Helix-turn-helix. The part shows a simple drawing and a ribbon model diagram of the helix-turn-helix D N A-binding protein. The simple model of this structure, at the top, shows 2 helixes modeled as cylinders both sandwiching the turn portion, which is drawn as a sinusoidal wave. The ribbon model diagram below shows the helix-turn-helix dimer protein interacting with the D N A double helix. One helix of the protein aligns with the turns of the D N A on both the sides and the turn part runs through the interior of the D N A helix.

Part b is labeled Zinc fingers. The part shows a simple drawing and a ribbon model diagram of the zinc finger D N A-binding protein. The simple model, at the top, looks like 2 hairpin structures or 2 fingers, each one having 2 alpha helixes. In the middle of the hairpin is a zinc ion, positioned so that the 2 alpha helixes pinch together. The ribbon model diagram below shows the zinc finger interacting with the D N A double helix. The alpha helixes at the top of the zinc finger project into the major groove of the D N A helix and the rest of the protein is alongside the D N A double helix. The zinc ions lie directly underneath the fingers.

Part c is labeled Leucine zipper. The part shows a simple drawing and a ribbon model diagram of the leucine zipper D N A-binding protein. The simple model, at the top, shows a linear protein containing an alpha helix, a straight line, and then a second longer alpha helix containing leucine side chains. The ribbon model diagram below shows the leucine zipper forming dimers and their interacting with the D N A double helix. The first alpha helix of the protein runs through the center of the D N A double helix and the second alpha helix of the protein has leucine that line up on one side of the protein. This portion of the protein forms dimers, so that leucines form a channel in the middle of the dimer.

TABLE 16.1 Common DNA-binding motifs

Motif	Location	Characteristics	Binding

			Site in DNA
Helix-turn-helix	Bacterial regulatory proteins; related motifs in eukaryotic proteins	Two alpha helices	Major groove
Zinc finger	Eukaryotic regulatory and other proteins	Loop of amino acids with zinc at base	Major groove
Steroid receptor	Eukaryotic proteins	Two perpendicular alpha helices with zinc surrounded by four cysteines	Major groove and DNA backbone
Leucine zipper	Eukaryotic transcription factors	Helix of leucine and a basic arm; two leucines interdigitate	Two adjacent major grooves
Helix-loop-helix	Eukaryotic proteins	Two alpha helices separated by a	Major groove

		loop of amino acids	
Homeodomain	Eukaryotic regulatory proteins	Three alpha helices	Major groove

CONCEPTS

Regulatory proteins that bind DNA have common motifs that interact with the double-helical structure of DNA.

✔ CONCEPT CHECK 3

How do amino acids in DNA-binding proteins interact with DNA?

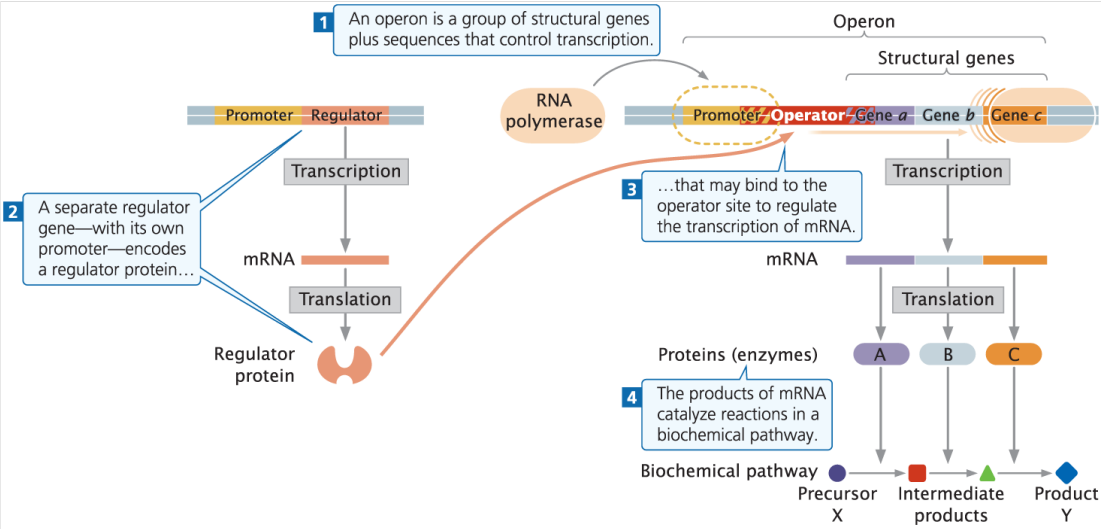
a. By forming covalent bonds with DNA bases
b. By forming hydrogen bonds with DNA bases
c. By forming covalent bonds with DNA sugars

16.2 Transcription in Bacterial Cells Is Regulated by Operons

A significant difference between bacterial and eukaryotic gene control lies in the organization of functionally related genes. As we saw in the introduction to this chapter, many bacterial genes that have related functions are clustered together and under the control of a single promoter. These genes are often transcribed together into a single mRNA molecule. A group of bacterial structural genes that are transcribed together, along with their promoter and additional sequences that control their transcription, is called an **operon**. The operon regulates the expression of the structural genes by controlling transcription, which, in bacteria, is usually the most important level of gene regulation.

Operon Structure

The organization of a typical operon is illustrated in **Figure 16.3**. At one end of the operon is a set of structural genes, shown in Figure 16.3 as gene *a*, gene *b*, and gene *c*. These structural genes are transcribed into a single mRNA, which is translated to produce enzymes A, B, and C. These enzymes carry out a series of biochemical reactions that convert precursor molecule X into product Y. The transcription of structural genes *a*, *b*, and *c* is under the control of a single promoter, which lies upstream of the first structural gene. RNA polymerase binds to the promoter and then moves downstream, transcribing the structural genes.



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16.3 An operon is a single transcription unit that includes a series of structural genes, a promoter, and an operator.

Description

The illustration shows formation of a regulator protein on the left. A double strand is present at the top with promoter region on the left and regulator on the right. A caption reads, A separate regulator gene—with its own promoter—encodes a regulator protein. Transcription occurs and a linear m R N A is formed below. Translation occurs and a regulator protein is formed below. The illustration also shows an operon with its components on the top right. The components from left to right are as follows: the promoter, operator, gene small A, small B, and small C. Genes small A, small B, and small C are collectively labeled as structural genes. The operator of the operon overlaps with the promoter on the left and gene small A on the right. An R N A polymerase is present on the left, and an arrow emerging from it points to the promoter. An oval shaped dotted line shows the R N A polymerase will bind over the promoter and operator region and will gradually move towards the right end of the operon. A caption reads, An operon is a group of structural genes plus sequences that control transcription. The regulator protein formed in translation has an arrow that points to the operator. A caption reads, that may bind to the operator site to regulate the transcription of m R N A. Transcription occurs below and an m R N A is obtained with genes small A, small B, and small C from left to right. Translation occurs and three proteins (enzymes) are formed below from left to right: Big A corresponding to gene small A, big B corresponding to gene small B, and big C corresponding to gene small C. A caption reads, The products of m R N A catalyze reactions in a biochemical pathway. A biochemical pathway is shown below. Pprecursor big X is converted to intermediate in the presence of protein big A. This intermediate is converted to another intermediate, in the presence of protein big B. The second intermediate is converted to product big Y , in the presence of protein big C.

A **regulator gene** helps control the expression of the structural genes of the operon by increasing or decreasing their transcription. Although it affects operon function, the regulator gene is not considered part of the operon. The regulator gene has its own promoter and is transcribed into a short mRNA, which is translated into a small

protein. This **regulator protein** can bind to a region of the operon called the **operator** and affect whether transcription can take place. The operator usually overlaps the 3′ end of the promoter and sometimes the 5′ end of the first structural gene (see Figure 16.3).

CONCEPTS

Functionally related genes in bacterial cells are frequently clustered together in a single transcription unit termed an operon. A typical operon includes several structural genes, a promoter for those structural genes, and an operator to which the product of a regulator gene binds.

✔ CONCEPT CHECK 4

What is the difference between a structural gene and a regulator gene?

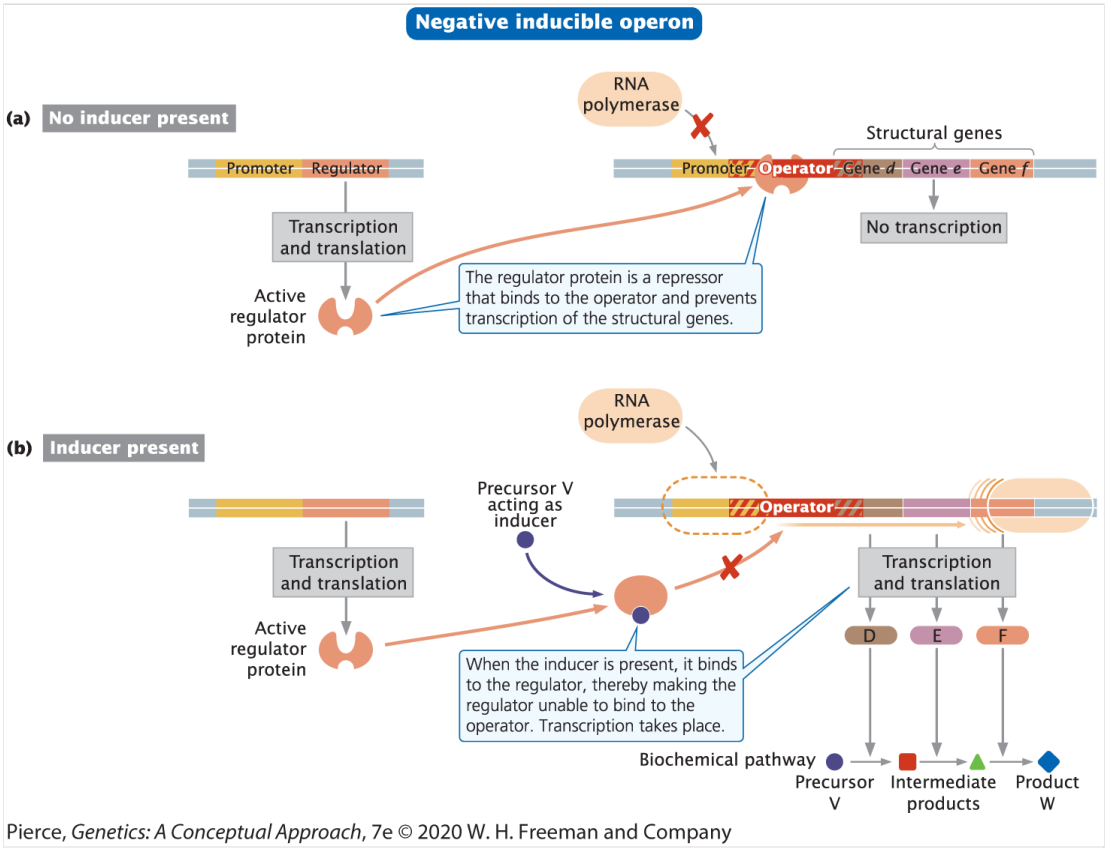
a. Structural genes are transcribed into mRNA, but regulator genes aren't.
b. Structural genes have complex structures; regulator genes have simple structures.
c. Structural genes encode proteins that function in the structure of the cell; regulator genes carry out metabolic reactions.
d. Structural genes encode proteins used in metabolism or biosynthesis or cell structure; regulator genes control the transcription of structural genes.

Negative and Positive Control: Inducible and Repressible Operons

There are two types of transcriptional control: **negative control**, in which a regulatory protein is a repressor, binding to DNA and inhibiting transcription; and **positive control**, in which a regulatory protein is an activator, stimulating transcription. Operons can also be either inducible or repressible. **Inducible operons** are those in which transcription is normally off (not taking place); something must happen to induce transcription, or turn it on. **Repressible operons** are those in which transcription is normally on (taking place); something must happen to repress transcription, or turn it off. In the next few sections, we will consider several varieties of these basic control mechanisms.

NEGATIVE INDUCIBLE OPERONS

The regulator gene for a negative inducible operon encodes an active *repressor* protein that readily binds to the operator (Figure 16.4a). Because the operator site overlaps the promoter site, the binding of this protein to the operator physically blocks the binding of RNA polymerase to the promoter and prevents transcription. For transcription to take place, something must happen to prevent the binding of the repressor to the operator. This type of system is said to be *inducible* because transcription is normally off (inhibited) and must be turned on (induced).



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16.4 Some operons are inducible.

Description

Text at the top center of the illustration reads, negative inducible operon. Part a is labeled no inducer present. The part shows formation of a regulator protein on the left. A double strand is present at the top with promoter region on the left and regulator on the right. Transcription and translation occurs and an active regulator protein is formed below. Part a also shows an operon with its components on the top right. The components from left to right are as follows: the promoter, operator, gene small D, small E, and small F. Genes small D, small E, and small F are

collectively labeled as structural genes. An R N A polymerase is present on the left and an arrow points to the promoter with an X over it. The active regulator protein formed on the left binds at the operator. A caption reads, the regulator protein is a repressor that binds to the operator and prevents transcription of the structural genes. A downward arrow below the operon indicates no transcription occurs.

Part b below is labeled Inducer present. The part shows formation of a regulator protein on the left. A double strand is present at the top with promoter region on the left and regulator on the right. Transcription and translation occurs and an active regulator protein is formed below. Part b also shows an operon with its components on the top right. The components from left to right are as follows: the promoter, operator, gene small D, small E, and small F. An R N A polymerase is present on the left and an arrow points to the promoter. An oval shaped dotted line shows the R N A polymerase will bind over the promoter and operator region and will gradually move towards the right end of the operon. A small precursor V acting as inducer gets attached to active regulator protein and an arrow emerging from this points to operator. A cross is marked over this arrow. A caption reads, when the inducer is present, it binds to the regulator, thereby making the regulator unable to bind to the operator. Transcription and translation occurs and proteins (enzymes) are formed below from left to right: Big D corresponding to gene small D, big E corresponding to gene small E, and big F corresponding to gene small F. A biochemical pathway is shown below. A precursor big V is converted to intermediate in the presence of protein big D. This intermediate is converted to another intermediate, in the presence of protein big E. The second intermediate is converted to product big W in the presence of protein big F.

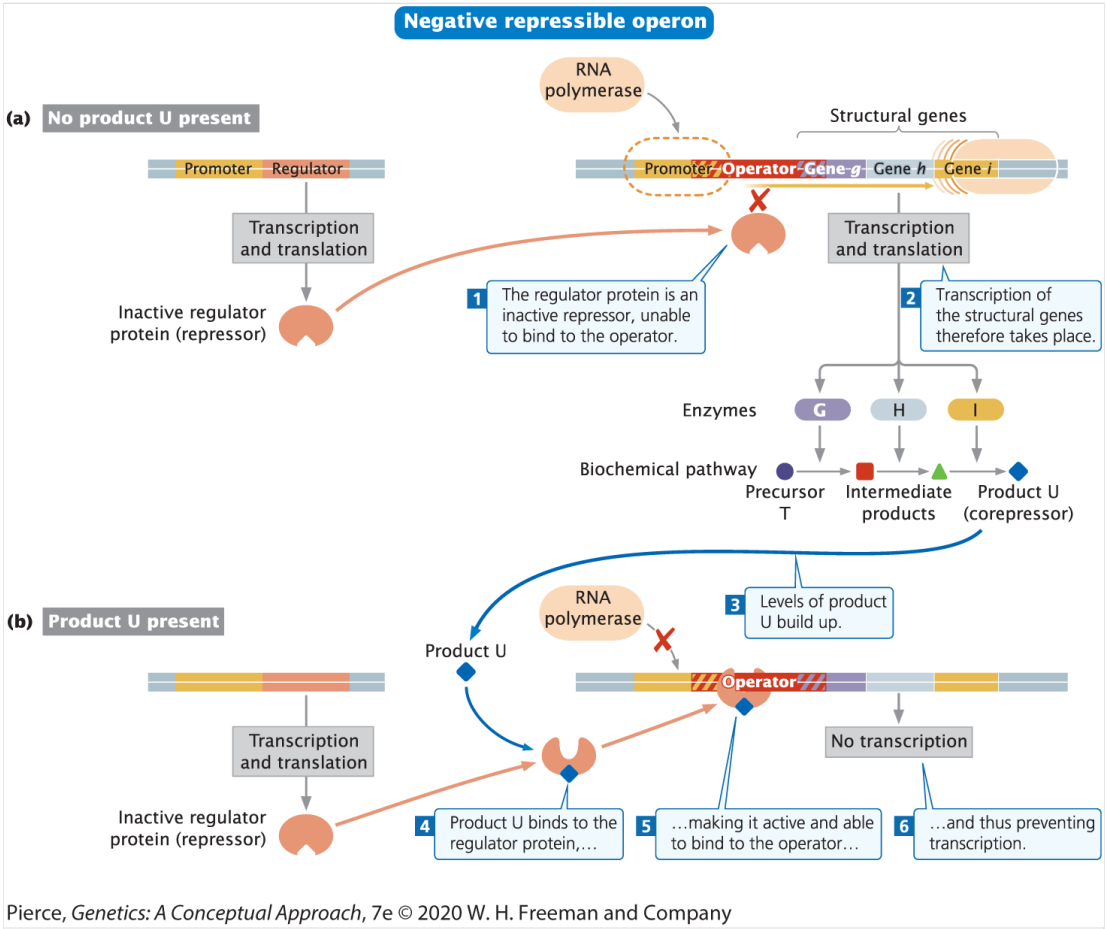
Transcription of a negative inducible operon is turned on when a small molecule called an **inducer** is present and binds to the repressor protein (**Figure 16.4b**). Regulatory proteins frequently have two binding sites: one that binds to DNA and another that binds to a small molecule such as an inducer. The binding of the inducer (precursor V in Figure 16.4b) alters the shape of the repressor, preventing it from binding to DNA. Proteins such as this repressor, which change shape upon binding to another molecule, are called **allosteric proteins**.

When the inducer is absent, the repressor binds to the operator, the structural genes are not transcribed, and enzymes D, E, and F (which metabolize precursor V) are not synthesized (see Figure 16.4a). This mechanism is an adaptive one: because no precursor V is present, synthesis of the enzymes would be wasteful because they would have no substrate to metabolize. As soon as precursor V becomes available, some of it binds to the repressor, rendering the repressor inactive and unable to bind to the operator. RNA polymerase can now bind to the promoter and transcribe the structural genes. The resulting mRNA is then translated into enzymes D, E, and F, which convert substrate V into product W (see Figure 16.4b). So, an operon with negative inducible control regulates the synthesis of the enzymes economically: the enzymes are synthesized only when their substrate (V) is available.

Inducible operons usually control proteins that carry out degradative processes—proteins that break down molecules. For these types of proteins, inducible control makes sense because the proteins are not needed unless the substrate (which is broken down by the proteins) is present.

NEGATIVE REPRESSIBLE OPERONS

Some operons with negative control are *repressible*, meaning that transcription *normally* takes place and must be turned off, or repressed. The regulator protein that acts on this type of operon is also a repressor, but it is synthesized in an *inactive* form that cannot by itself bind to the operator. Because no repressor is bound to the operator, RNA polymerase readily binds to the promoter, and transcription of the structural genes takes place (Figure 16.5a).



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16.5 Some operons are repressible.

Description

Text at the top center reads, Negative repressible operon. Part a, at the top, is labeled No product U present. Part a shows formation of an inactive regulator protein (repressor) on the left. A double strand is present at the top with promoter region on the left and regulator on the right. Transcription and translation occurs and an inactive regulator protein is formed below. Part also shows an operon with its components on the top right. The components from left to right are as follows: the promoter, operator, gene small G, small H, and small I. Genes small G, small H, and small I are collectively labeled as structural genes. An R N A polymerase is present on the left and an arrow points to the promoter. An oval shaped dotted line shows the R N A polymerase will bind over the promoter and operator region and will gradually move towards the right end of the operon. The inactive regulator protein does not bind to the operator. A caption reads, The regulator protein is an inactive repressor, unable to bind to the operator. Transcription and translation occurs and proteins (enzymes) are formed below from left to right: Big G corresponding to gene small G, big H corresponding to gene small H, and big I corresponding to gene small I. A caption reads, Transcription of the structural genes therefore takes place. A biochemical pathway is shown below. A precursor big T is converted to intermediate in the presence of protein big G. This intermediate is converted to another intermediate, in the presence of protein big H. The second intermediate is converted to product big U in the presence of protein big I.

Part b is labeled Product U present. The part shows formation of an inactive regulator protein (repressor) on the left. A double strand is present at the top with promoter region on the left and regulator on the right. Transcription and translation occurs and an inactive regulator protein is formed below. Part also shows an operon with its components on the top right. The components from left to right are as follows: the promoter, operator, gene small G, small H, and small I. An R N A polymerase is present on the left and an arrow points to the promoter. This arrow has an X over it. Product big U attaches to the repressor formed and binds to the operator. A caption reads, Levels of product big U build up. Product big U binds to the regulator protein, ellipses. Ellipses making it active and able to bind to the operator ellipses. Ellipses and thus preventing transcription. A downward arrow from the operon shows no transcription takes place.

To turn transcription off, something must happen to make the repressor active. When a small molecule called a **corepressor** is present, it binds to the repressor and makes it capable of binding to the operator. In the example illustrated (see Figure 16.5a), the product (U) of the metabolic reaction controlled by the operon is the corepressor. As long as the level of product U is high, it is available to bind to the repressor and activate it, preventing transcription (**Figure 16.5b**). With the operon repressed, enzymes G, H, and I are not synthesized, and no more U is produced from precursor T. However, when all of product U is used up, the repressor is no longer activated by product U and cannot bind to the operator. The inactivation of the repressor allows the transcription of the structural genes and the synthesis of enzymes G, H, and I, resulting in the conversion of precursor T into product U. Like inducible operons, repressible operons are economical: the proteins they encode are synthesized only as needed.

Repressible operons usually control proteins that carry out the biosynthesis of molecules needed in the cell, such as amino acids. For these types of operons, repressible control makes sense because the product produced by the proteins is always needed by the cell. Thus, these operons are normally on and are turned off only when there are adequate amounts of the product already present.

Note that both the inducible and the repressible operon systems that we have just considered are forms of negative control, in which the regulator protein is a repressor.
› **TRY** PROBLEM 12

POSITIVE CONTROL

In positive transcriptional control, the regulatory protein involved is an activator: it binds to DNA (usually at a site other than the operator) and stimulates transcription. For example, in the case of the *lac* operon, the catabolite activator protein (CAP) binds to the promoter and increases the efficiency with which RNA polymerase can bind the promoter and transcribe the structural genes (as we'll see later in this section).

THINK-PAIR-SHARE Question 3

CONCEPTS

There are two basic types of transcriptional control: negative and positive. In negative control, when a regulator protein (repressor) binds to DNA, transcription is inhibited; in positive control, when a regulator protein (activator) binds to DNA, transcription is stimulated. Some operons are inducible: their transcription is normally off and must be turned on. Other operons are repressible: their transcription is normally on and must be turned off.

✔ CONCEPT CHECK 5

The regulator protein that acts on a negative repressible operon is synthesized as

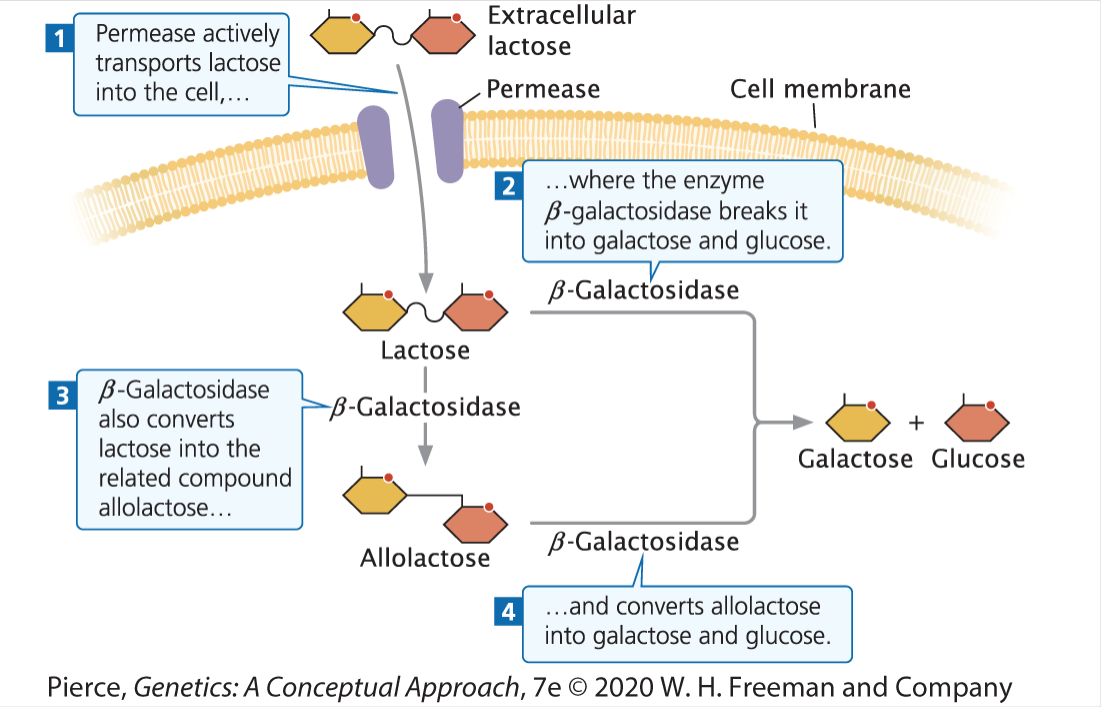
a. an active activator.
b. an inactive activator.
c. an active repressor.
d. an inactive repressor.

The *lac* Operon of *E. coli*

François Jacob and Jacques Monod first described the "operon model" for the genetic control of lactose metabolism in *E. coli* in 1961 (see introduction to this chapter). Their work, and subsequent research on the genetics of lactose metabolism, established the operon as the basic unit of transcriptional control in bacteria. Despite the fact that at the time, no methods were available for determining nucleotide sequences, Jacob and Monod deduced the structure of the operon *genetically* by analyzing the interactions of mutations that interfered with the normal regulation of lactose metabolism. We examine the effects of some of these mutations after seeing how the *lac* operon regulates lactose metabolism.

LACTOSE METABOLISM

Lactose is a major carbohydrate found in milk; it can be metabolized by *E. coli* bacteria that reside in the mammalian gut. Lactose does not easily diffuse across the *E. coli* cell membrane and must be actively transported into the cell by the protein lactose permease (**Figure 16.6**). To use lactose as an energy source, *E. coli* must then break it into glucose and galactose, a reaction catalyzed by the enzyme *β*-galactosidase. This enzyme can also convert lactose into allolactose, a compound that plays an important role in regulating lactose metabolism. A third enzyme, thiogalactoside transacetylase, is also produced by the *lac* operon, but its function in lactose metabolism isn't clear. One possible function is detoxification, preventing the accumulation of thiogalactosides that are transported into the cell along with lactose by permease.



16.6 Lactose, a major carbohydrate found in milk, consists of two six-carbon sugars linked together.

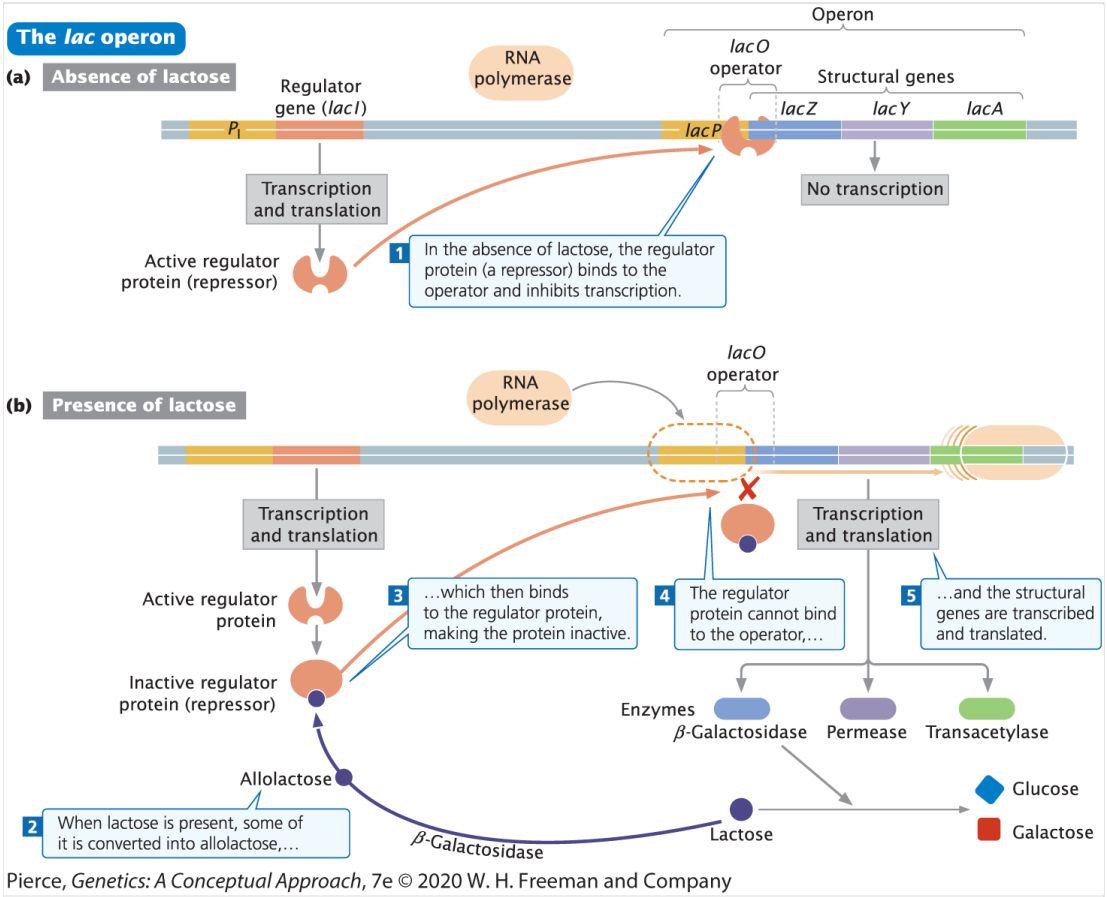
Description

The illustration shows the structure of lactose. Two six carbon sugars are linked together are labeled extracellular lactose. The sugar molecules have a linkage between the C 1 of the first and C 4 of the second. A bilipid layer below the lactose is labeled cell membrane. An opening has two permease proteins on both the sides and that is the point of entry of extracellular lactose. A caption reads, Permease actively transports lactose into the cell where the enzyme Beta-galactosidase breaks it into galactose and glucose. Lactose enters the cell and Beta-galactosidase converts it into galactose and glucose shown on the right. Beta-galactosidase converts it into allolactose shown below, where there is a linkage between C 1 and C 5. A caption reads, Beta-Galactosidase also converts lactose into the related compound allolactose. Allolactose is also converted to galactose and glucose, after Beta-galactosidase breaks it again. A caption reads, and converts allolactose into galactose and glucose.

REGULATION OF THE *lac* OPERON

The *lac* operon of *E. coli* is an example of a negative inducible operon. The enzymes β-galactosidase, permease, and transacetylase are encoded by adjacent structural genes in the *lac* operon (Figure 16.7a) and have a common promoter (*lacP* in Figure 16.7a). β-Galactosidase is encoded by the *lacZ* gene, permease by the *lacY* gene, and transacetylase by the *lacA* gene. When lactose is absent from the medium in which *E.*

coli grows, few molecules of each protein are produced. If lactose is added to the medium and glucose is absent, the rate of synthesis of all three proteins simultaneously increases about a thousandfold within 2 to 3 minutes. This boost in protein synthesis, which results from the simultaneous transcription of *lacZ*, *lacY*, and *lacA*, exemplifies **coordinate induction**, the simultaneous synthesis of several proteins stimulated by a specific molecule, the inducer (Figure 16.7b).



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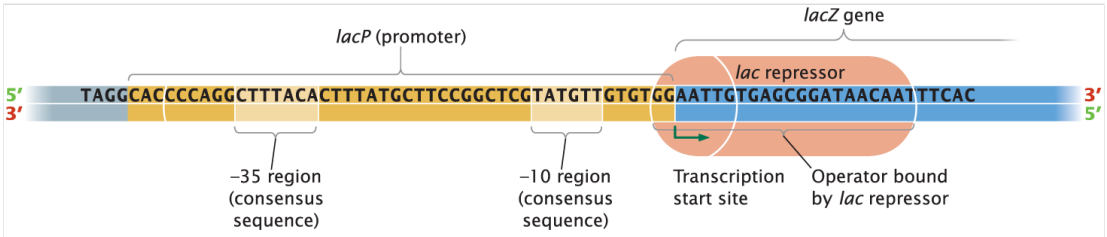
16.7 The *lac* operon regulates lactose metabolism.

Description

Part a is labeled absence of lactose. A lac operon shows the following components from left to right: P subscript I, Regulator gene (lac big I), lac big P, lac big Z, lac big Y, and lac big A. Lac big Z, lac big Y, and lac big A are collectively labeled as structural genes. An R N A polymerase is present at the top of the operon. Transcription and translation occurs for the regulator gene and an active regulator protein (repressor) is formed below. This repressor binds at the right end of the lac big P gene. Lac big O operator is the region with the repressor bound to the lac big P and lac big Z gene. A caption reads, In the absence of lactose, the regulator protein (a repressor) binds to the operator and inhibits transcription. No transcription takes place.

Part b below is labeled presence of lactose. A lac operon shows the following components from left to right: P subscript I, Regulator gene (lac big I), lac big P, lac big Z, lac big Y, and lac big A. An R N A polymerase is present at the top of the operon. Transcription and translation occur for lac promoter and an active regulator protein is formed below. An oval shaped dotted line shows R N A polymerase binds to lac big P gene and would love forward to the right. Transcription and translation occur for the structural genes and the following enzymes are obtained below: Beta-Galactosidase, Permease, and Transacetylase. Lactose is present below. Beta galactosidase converts lactose into glucose and galactose. Lactose also converts to allolactose in the presence of Beta galactosidase. A caption reads, When lactose is present, some of it is converted into allolactose. Allolactose binds to active regulator protein making it inactive (repressor). A caption reads, which then binds to the regulator protein, making the protein inactive. An arrow from inactive regulator protein shows it cannot any longer bind to the operator. A caption reads, The regulator protein cannot bind to the operator. The transcription and translation of structural genes occur. A caption reads, and the structural genes are transcribed and translated.

Although lactose might appear to be the inducer in this case, allolactose is actually responsible for induction. Upstream of *lacP* is a regulator gene, *lacI*, which has its own promoter (P_I). The *lacI* gene is transcribed into a short mRNA that is translated into a repressor. The repressor consists of four identical polypeptides and has two types of binding sites; one type of site binds to allolactose and the other binds to DNA. In the absence of lactose (and, therefore, allolactose), the repressor binds to the *lac* operator *lacO* (see Figure 16.7a). Jacob and Monod mapped the operator to a position adjacent to the *lacZ* gene; nucleotide sequencing has demonstrated that the operator actually overlaps the 3′ end of the promoter and the 5′ end of *lacZ* (**Figure 16.8**).



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16.8 In the *lac* operon, the operator overlaps the 3′ end of promoter and the 5′ end of the first structural gene.

Description
The illustration shows a double strand with the top strand running from 5 prime to 3 prime and bottom strand running from 3 prime to 5 prime. The following nucleotides are present on the top strand, from left to right: T, A, G, G, lac big P (promoter) starts C, A, C, C, C, C, A, G, G, negative 35 region consensus sequence begins C, T, T, T, A, C, A, negative 35 region consensus sequence ends C, T, T, T, A, T, G, C, T, T, C, C, G, G, C, T, C, G, negative 10 region consensus sequence begins T, A, T, G, T, T negative 10 region consensus sequence ends, G, T, G, T lac repressor

region starts, G, G, lac big P (promoter) ends transcription start site, and lac big Z gene starts A, A , T, T, G, T, G, A, G, C, G, G, A, T, A, A, C, A , A, T, lac repressor region ends T, T, C, A, C.

When the repressor is bound to the operator, the binding of RNA polymerase is blocked, and transcription is prevented. When lactose is present, some of it is converted into allolactose, which binds to the repressor and causes the repressor to be released from the operator. RNA polymerase then binds to the promoter and moves down the DNA molecule, transcribing the structural genes. In the presence of lactose, then, the repressor is inactivated, the binding of RNA polymerase is no longer blocked, the transcription of *lacZ*, *lacY*, and *lacA* takes place, and the *lac* proteins are produced.

Have you spotted the flaw in the explanation just given for the induction of *lac* protein synthesis? You might recall that permease is required to transport lactose into the cell. If the *lac* operon is repressed and no permease is being produced, how does lactose get into the cell to inactivate the repressor and turn on transcription? Furthermore, the inducer is actually allolactose, which must be produced from lactose by *β*-galactosidase. If *β*-galactosidase production is repressed, how can lactose metabolism be induced?

The answer is that repression never *completely* shuts down transcription of the *lac* operon. Even with an active repressor bound to the operator, there is a low level of transcription, and a few molecules of *β*-galactosidase, permease, and transacetylase are synthesized. When lactose appears in the medium, the permease that is present transports a small amount of lactose into the cell. There, the few molecules of *β*-galactosidase that are present convert some of the lactose into allolactose, which then induces transcription.

Several compounds related to allolactose can also bind to the *lac* repressor and induce transcription of the *lac* operon. One such inducer is isopropylthiogalactoside (IPTG). Although IPTG inactivates the repressor and allows the transcription of *lacZ*, *lacY*, and *lacA*, this inducer is not metabolized by *β*-galactosidase; for this reason, IPTG is often used in research to examine the effects of induction independent of metabolism.

THINK-PAIR-SHARE Question 4

CONCEPTS

The *lac* operon of *E. coli* controls the transcription of three genes needed in lactose metabolism: the *lacZ* gene, which encodes β-galactosidase; the *lacY* gene, which encodes lactose permease; and the *lacA* gene, which encodes thiogalactoside transacetylase. The *lac* operon is a negative inducible operon: a regulator gene produces a repressor that binds to the operator and prevents the transcription of the structural genes. The presence of allolactose inactivates the repressor and allows the transcription of the *lac* operon.

✔ CONCEPT CHECK 6

In the presence of allolactose, the *lac* repressor

a. binds to the operator.
b. binds to the promoter.
c. cannot bind to the operator.
d. binds to the regulator gene.

lac Mutations

Jacob and Monod worked out the structure and function of the *lac* operon by analyzing mutations that affected lactose metabolism. To help define the roles of the different components of the operon, they used **partial-diploid** strains of *E. coli*. The cells of these strains possessed two different DNA molecules: the full bacterial chromosome and an extra piece of DNA. Jacob and Monod created these strains by allowing conjugation to take place between two bacteria. In conjugation, a small circular piece of DNA (the F plasmid) is transferred from one bacterium to another (see Chapter 9). The F plasmid used by Jacob and Monod contained the *lac* operon, so the recipient bacterium became partly diploid, possessing two copies of the *lac* operon. By using different combinations of mutations on the bacterial and plasmid DNA, Jacob and Monod determined that some parts of the *lac* operon are cis acting (able to control the expression of genes only on the same piece of DNA), whereas other parts are trans acting (able to control the expression of genes on other DNA molecules).

STRUCTURAL-GENE MUTATIONS

Jacob and Monod first discovered some mutant strains that had lost the ability to synthesize either *β*-galactosidase or permease. (They did not study in detail the effects of mutations on the transacetylase enzyme, so transacetylase will not be considered here.) The mutations in those mutant strains mapped to the *lacZ* or *lacY* structural genes and altered the amino acid sequences of the proteins encoded by the genes. These mutations clearly affected the *structure* of the proteins but not the regulation of their synthesis.

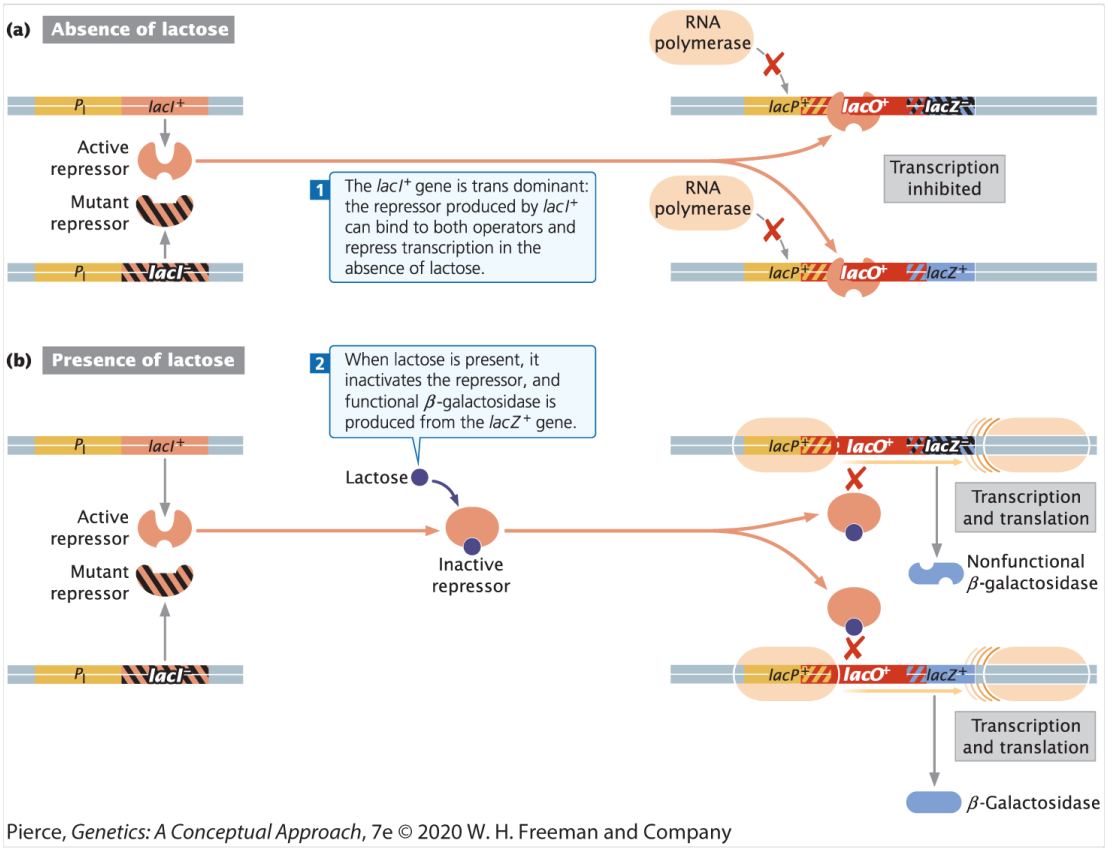
Through the use of partial diploids, Jacob and Monod were able to establish that mutations of the *lacZ* and *lacY* genes were independent and usually affected only the product of the gene in which the mutation occurred. Partial diploids with $lacZ^+\ lacY^-$ on the bacterial chromosome and $lacZ^-\ lacY^+$ on the plasmid functioned normally, producing *β*-galactosidase and permease in the presence of lactose. (The genotype of a partial diploid is written by separating the genes on each DNA molecule with a slash: $lacZ^+\ lacY^-/lacZ^-\ lacY^+$.) In these partial diploids, a single functional *β*-galactosidase gene ($lacZ^+$) was sufficient to produce *β*-galactosidase; whether the functional *β*-galactosidase gene was coupled to a functional ($lacY^+$) or a defective ($lacY^-$) permease gene made no difference. The same was true of the $lacY^+$ gene.

REGULATOR-GENE MUTATIONS

Jacob and Monod also isolated mutations that affected the *regulation* of protein production. Mutations in the *lacI* gene affect the production of both *β*-galactosidase and permease because the genes for both proteins are in the same operon and are regulated coordinately by the *lac* repressor protein.

Some of these mutations were constitutive, causing the *lac* proteins to be produced all the time, whether lactose was present or not. Such mutations in the regulator gene were designated $lacI^-$. The construction of partial diploids demonstrated that a $lacI^+$ gene is dominant over a $lacI^-$ gene: a single copy of $lacI^+$ (genotype $lacI^+/lacI^-$) was sufficient to bring about normal regulation of protein production. Furthermore, $lacI^+$

restored normal control to an operon even if the operon was located on a different DNA molecule, showing that *lacI*$^+$ can be trans acting. A partial diploid with genotype *lacI*$^+$ *lacZ*$^-$/*lacI*$^-$ *lacZ*$^+$ functioned normally, synthesizing β-galactosidase only when lactose was present (Figure 16.9). In this strain, the *lacI*$^+$ gene on the bacterial chromosome was functional, but the *lacZ*$^-$ gene was defective; on the plasmid, the *lacI*$^-$ gene was defective, but the *lacZ*$^+$ gene was functional. The fact that a *lacI*$^+$ gene could regulate a *lacZ*$^+$ gene located on a different DNA molecule indicated to Jacob and Monod that the *lacI*$^+$ gene product was able to operate on either the plasmid or the chromosome.



16.9 The partial diploid *lacI*$^+$ *lacZ*$^-$/*lacI*$^-$ *lacZ*$^+$ produces β-galactosidase only in the presence of lactose because the *lacI gene* is trans dominant.

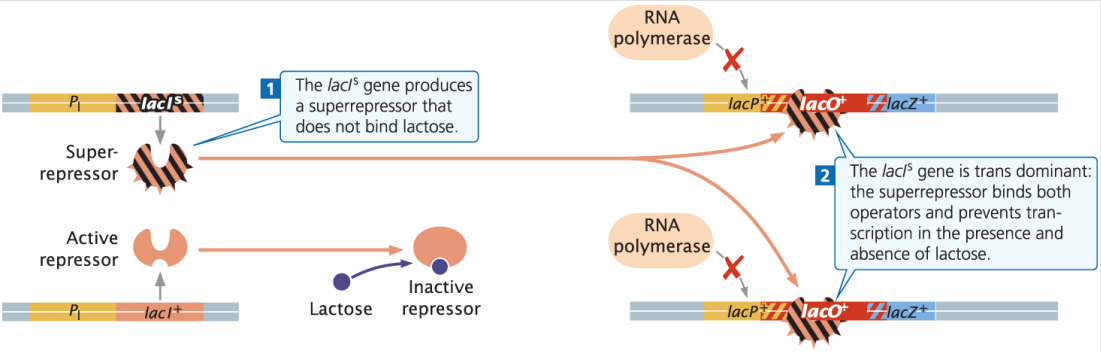
Description

Part a at the top shows Absence of lactose. A double strand, at the top left, shows P subscript I and lac big I superscript positive gene. It leads to formation of an active repressor below. Another double strand below has P subscript I and lac big I superscript negative gene. It leads to the formation of a mutant repressor above it. An operon is present to the right of first double strand and another operon is present to the right of second double strand. The operon at the top has the following from left to right: lac big P superscript positive, lac big O superscript positive, and

lac big Z superscript negative. An R N A polymer is present above this operon with an arrow pointing to the lac big P superscript positive. The arrow has a cross mark. The operon below has the following from left to right: lac big P superscript positive, lac big O superscript positive, and lac big Z superscript positive. An R N A polymer is present above this operon with an arrow pointing to the lac big P superscript positive. The arrow has an X over it. The active repressor formed on the left binds to lac big O superscript positive for both strands on the right. A caption reads, The lac big I superscript positive gene is trans dominant: the repressor produced by lac big I superscript positive can bind to both operators and repress transcription in the absence of lactose. Transcription is inhibited.

Part b below shows presence of lactose. A double strand, at the top left, shows P subscript I and lac big I superscript positive gene. It leads to formation of an active repressor below. Another double strand below has P subscript I and lac big I superscript negative gene. It leads to the formation of a mutant repressor above it. An operon is present to the right of first double strand and another operon is present to the right of second double strand. The operon at the top has the following from left to right: lac big P superscript positive, lac big O superscript positive, and lac big Z superscript negative. An R N A polymer binds to the lac big P superscript positive and shows movement from left to right. The operon below has the following from left to right: lac big P superscript positive, lac big O superscript positive, and lac big Z superscript positive. An R N A polymer binds to the lac big P superscript positive and shows movement from left to right. The active repressor formed on the left binds to lactose and becomes inactive repressor. The inactive repressor now cannot bind to the lac big O superscript positive for both strands on the right. A caption reads, When lactose is present, it inactivates the repressor, and functional Beta-galactosidase is produced from the lac big Z superscript positive gene. The lac big Z superscript negative gene, of the above operon, produces Nonfunctional Beta-galactosidase below after transcription and translation occur. The lac big Z superscript positive gene, of the below operon, produces Beta-galactosidase below after transcription and translation occur.

Some *lacI* mutations isolated by Jacob and Monod prevented transcription from taking place even in the presence of lactose. These mutations were referred to as superrepressors ($lacI^s$) because they produced defective repressor proteins that could not be inactivated by an inducer. A $lacI^s$ mutation produced a repressor with an altered inducer-binding site; consequently, the inducer was unable to bind to the repressor, and the repressor was always able to attach to the operator and prevent transcription of the *lac* genes. Superrepressor mutations were dominant over $lacI^+$; partial diploids with genotype $lacI^s\ lacZ^+\ lacY^+ / lacI^+\ lacZ^+\ lacY^+$ were unable to synthesize either β-galactosidase or permease, whether or not lactose was present (Figure 16.10).



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16.10 The partial diploid *lacI*^{S} *lacZ*$^+$/*lacI*$^+$ *lacZ*$^+$ fails to produce *β*-galactosidase in the presence and absence of lactose because the *lacI*^{S} gene encodes a superrepressor.

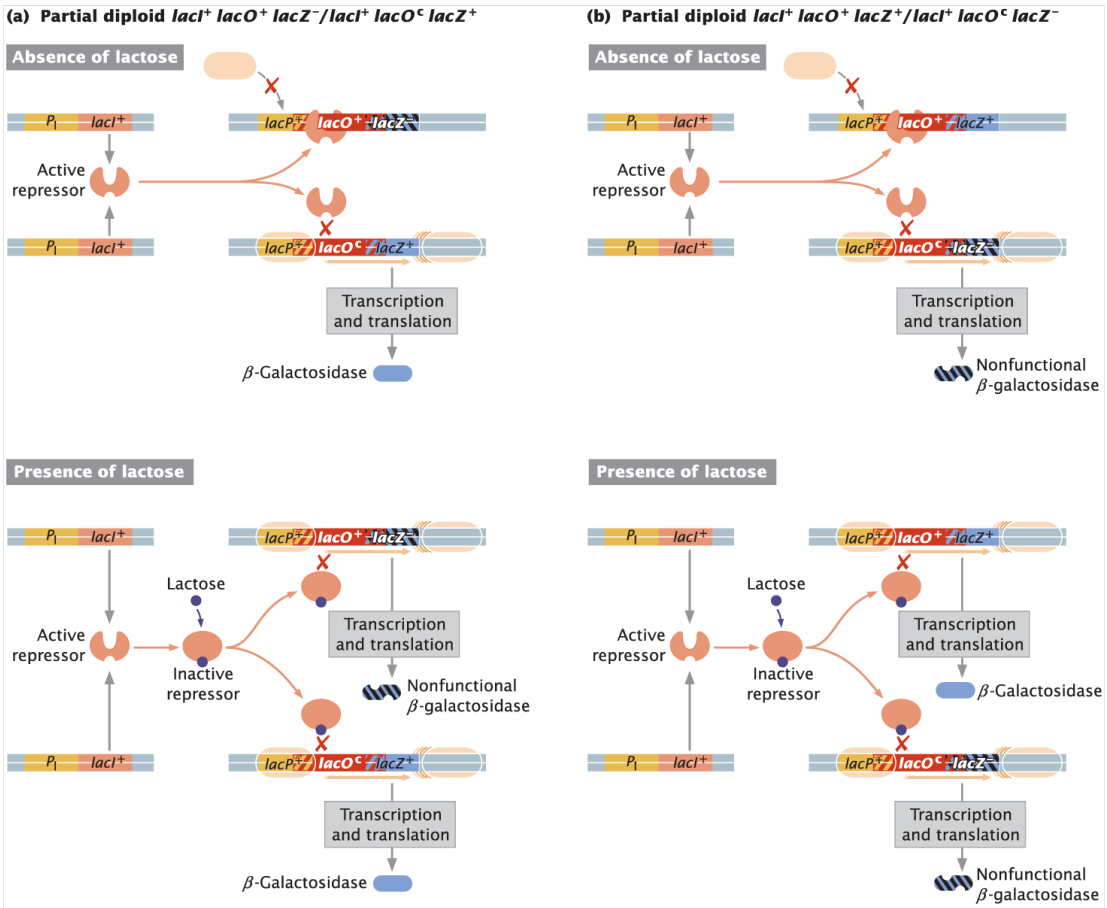
Description

A double strand at the top left shows P subscript I and lac big I superscript small S gene. It leads to formation of a super repressor below. Another double strand below has P subscript I and lac big I superscript positive gene. It leads to the formation of an active repressor above it. An operon is present to the right of first double strand and another operon is present to the right of second double strand. The operon at the top has the following from left to right: lac big P superscript positive, lac big O superscript positive, and lac big Z superscript positive. An R N A polymer is present above and an arrow with an X over it points to the lac big P superscript positive. The operon below has the following from left to right: lac big P superscript positive, lac big O superscript positive, and lac Z superscript positive. An R N A polymer is present above and an arrow with an X over it points to the lac big P superscript positive. The super repressor formed on the left binds to the lac big O superscript positive for both strands on the right. A caption reads, The lac big I superscript small S gene produces a super repressor that does not bind lactose. The lac big I superscript small S gene is trans dominant: the super repressor binds both operators and prevents transcription in the presence and absence of lactose. A lactose binds to the active repressor formed on the bottom left and makes it inactive.

OPERATOR MUTATIONS

Jacob and Monod mapped another class of constitutive mutations to a site adjacent to *lacZ*. These mutations occurred at the operator and were referred to as *lacO*^{c} (*O* stands for “operator” and c—for “constitutive”). The *lacO*^{c} mutations altered the sequence of DNA at the operator so that the repressor protein was no longer able to bind to it. A partial diploid with genotype *lacI*$^+$ *lacO*^{c} *lacZ*$^+$ / *lacI*$^+$ *lacO*$^+$ *lacZ*$^+$ exhibited constitutive synthesis of *β*-galactosidase, indicating that *lacO*^{c} is dominant over *lacO*$^+$.

Analysis of other partial diploids showed that the *lacO* gene is cis acting, affecting only genes on the same DNA molecule. For example, a partial diploid with genotype $lacI^+$ $lacO^+$ $lacZ^-$/$ilacI^+$ $lacO^c$ $lacZ^+$ was constitutive, producing β-galactosidase in the presence or absence of lactose (**Figure 16.11a**), but a partial diploid with genotype $lacI^+$ $lacO^+$ $lacZ^+$ /$lacI^+$ $lacO^c$ $lacZ^-$ produced β-galactosidase only in the presence of lactose (**Figure 16.11b**). In the constitutive partial diploid ($lacI^+$ $lacO^+$ $lacZ^-$ /$lacI^+$ $lacO^c$ $lacZ^+$, see Figure 16.11a), the $lacO^c$ mutation and the functional $lacZ^+$ gene were present on the same DNA molecule, but in $lacI^+$ $lacO^+$ $lacZ^+$/$lacI^+$ $lacO^c$ $lacZ^-$ (see Figure 16.11b), the $lacO^c$ mutation and the functional $lacZ^+$ gene were on different molecules. Thus, the *lacO* mutation affects only genes to which it is physically connected, as is true of all operator mutations. Such mutations prevent the binding of a repressor protein to the operator and thereby allow RNA polymerase to transcribe genes on the same DNA molecule. However, they cannot prevent a repressor from binding to normal operators on other DNA molecules. Watch **Animation 16.1** to observe the effects of different combinations of *lacI* and *lacO* mutations on the expression of the *lac* operon. **› TRY PROBLEM 23**



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16.11 Mutations in *lacO* are constitutive and cis acting. (a) The partial diploid *lacI*$^+$ *lacO*$^+$ *lacZ*$^-$/ *lacI*$^+$ *lacO*^{C} *lacZ*$^+$ is constitutive, producing β-galactosidase in the presence and absence of lactose. (b) The partial diploid *lacI*$^+$ *lacO*$^+$ *lacZ*$^+$/*lacI*$^+$ *lacO*^{C} *lacZ*$^-$ is inducible (produces β-galactosidase only when lactose is present), demonstrating that the *lacO* gene is cis acting.

Description

Part a on the right shows partial diploid lac big I superscript positive lac big O superscript positive lac big Z superscript negative slash lac big I superscript positive lac big O superscript small C lac big Z superscript positive.

Text reads absence of lactose. A double strand, at the top left, shows P subscript I and lac big I superscript positive gene. It leads to formation of an active repressor below. A double strand, below, shows P subscript I and lac big I superscript positive gene. It leads to formation of an active repressor above. An operon is present to the right of first double strand and another operon is present to the right of second double strand. The operon at the top has the following from left to right: lac big P superscript positive, lac big O superscript positive, and lac big Z superscript negative. An R N A polymer is present above and an arrow with an X over it points to the lac big P superscript positive. The operon below has the following from left to right: lac big P superscript positive, lac big O superscript C, and lac big Z superscript positive. The active repressor binds to the lac big O superscript positive on the above operon but not on the bottom operon. The R N A polymerase binds to the lac big P superscript positive of the top operon and moves from left to right. Transcription and translation occurs and beta-galactosidase is formed below.

Text at bottom left reads, presence of lactose. A double strand below shows P subscript I and lac big I superscript positive gene. It leads to the formation of an active repressor below. A double strand below shows P subscript I and lac big I superscript positive gene. It leads to formation of an active repressor above. An operon is present to the right of first double strand and another operon is present to the right of second double strand. The operon at the top has the following from left to right: lac big P superscript positive, lac big O superscript positive, and lac big Z superscript negative. The operon below has the following from left to right: lac big P superscript positive, lac big O superscript small C, and lac big Z superscript positive. The active repressor binds to a lactose and becomes inactive. The inactive repressor is unable to bind to the lac big O superscript positive of both the operons. The R N A polymerase binds to the lac big P superscript positive of both the operons and move from left to right. Transcription and translation occurs for both the operons: non functional beta galactosidase is formed for the above operon and beta-galactosidase is formed for below operon.

Part b on the right shows partial diploid lac big I superscript positive lac big O superscript positive lac big Z superscript positive slash lac big I superscript positive lac big O superscript small C lac big Z superscript negative.

Text reads absence of lactose. A double strand, below, shows P subscript I and lac big I superscript positive gene. It leads to formation of an active repressor below. A double strand, below, shows P subscript I and lac big I superscript positive gene. It leads to formation of an active repressor above. An operon is present to the right of first double strand and another operon is present to the right of second double strand. The operon at the top has the following from left to right: lac big P superscript positive, lac big O superscript positive, and lac big Z superscript positive. An R N A polymer is present above and an arrow with an X over it points to the lac big P superscript positive. The operon below has the following from left to right: lac big P superscript positive, lac big O superscript small C, and lac big Z superscript negative. The active repressor binds to the lac big O superscript positive on the above operon but not on the bottom operon. The R N A polymerase binds to the lac big P superscript positive on the below operon and moves from left to right. Transcription and translation occurs and non functional beta-galactosidase is formed below.

Text at bottom right reads, presence of lactose. A double strand, below, shows P subscript I and lac big I superscript positive gene. It leads to formation of an active repressor below. A double strand, below, shows P subscript I and lac big I superscript positive gene. It leads to formation of an active repressor above. An operon is present to the right of first double strand and another operon is present to the right of second double strand. The operon at the top has the following from left to right: lac big P superscript positive, lac big O superscript positive, and lac big Z superscript positive. The operon below has the following from left to right: lac big P superscript positive, lac big O superscript small C, and lac big Z superscript negative. The active repressor binds to a lactose and becomes inactive. The inactive repressor is unable to bind to the lac big O superscript positive of both the operons. The R N A polymerase binds to the lac big P superscript positive of both the operons and move from left to right. Transcription and translation occurs for both the operons: beta galactosidase is formed for the above operon and non functional beta-galactosidase is formed for below operon.

PROMOTER MUTATIONS

Mutations affecting lactose metabolism have also been isolated at the promoter; these mutations, designated *lacP*$^-$, interfere with the binding of RNA polymerase to the promoter. Because this binding is essential for the transcription of the structural genes,

E. coli strains with *lacP*$^-$ mutations don't produce *lac* proteins either in the presence or in the absence of lactose. Like operator mutations, *lacP*$^-$ mutations are cis acting and thus affect only genes on the same DNA molecule. The partial diploid *lacI*$^+$ *lacP*$^+$ *lacZ*$^+$/*lacI*$^+$ *lacP*$^-$ *lacZ*$^+$ exhibits normal synthesis of *β*-galactosidase, whereas *lacI*$^+$ *lacP*$^-$ *lacZ*$^+$/*lacI*$^+$ *lacP*$^+$ *lacZ*$^-$ fails to produce *β*-galactosidase whether or not lactose is present. The different types of mutations that occur in the *lac* operon are summarized in **Table 16.2**.

TABLE 16.2 Characteristics of *lac* operon mutations

Type	Location	Cis/Trans	Effect
Structural-gene mutations	*lacZ, lacY*	Affect only *lacZ* or *lacY*	Alter amino acid sequence of protein encoded by gene in which mutation occurs
Regulator-gene mutations	*lacI*	Trans	Affect transcription of structural genes
Operator mutations	*lacO*	Cis	Affect transcription of structural genes
Promoter mutations	*lacP*	Cis	Affect transcription of structural genes

WORKED PROBLEM

Make a table and, for the *E. coli* strains with the following *lac* genotypes, use a plus sign (+) to indicate the synthesis of *β*-galactosidase and permease and a minus sign (−) to indicate no synthesis of those proteins when lactose is absent and when it is present.

a. *lacI*$^+$ *lacP*$^+$ *lacO*$^+$ *lacZ*$^+$ *lacY*$^+$
b. *lacI*$^+$ *lacP*$^+$ *lacO*^{c} *lacZ*$^-$ *lacY*$^+$
c. *lacI*$^+$ *lacP*$^-$ *lacO*$^+$ *lacZ*$^+$ *lacY*$^-$

d. *lacI*$^{+}$ *lacP*$^{+}$ *lacO*$^{+}$ *lacZ*$^{-}$ *lacY*$^{-}$/*lacI*$^{-}$ *lacP*$^{+}$ *lacO*$^{+}$ *lacZ*$^{+}$ *lacY*$^{+}$

Solution Strategy

What information is required in your answer to the problem?

An indication of whether or not *β*-galactosidase and permease are produced by each genotype in the presence and in the absence of lactose by placement of a plus sign (+) or a minus sign (−) for each protein and condition in the table.

What information is provided to solve the problem?

The genotype of each strain.

Solution Steps

	Lactose absent		Lactose present	
Genotype of strain	***β*-Galactosidase**	**Permease**	***β*-Galactosidase**	**Permease**
a. *lacI*$^{+}$ *lacP*$^{+}$ *lacO*$^{+}$ *lacZ*$^{+}$ *lacY*$^{+}$	−	−	+	+
b. *lacI*$^{+}$ *lacP*+ *lacO*^{c} *lacZ*$^{-}$ *lacY*$^{+}$	−	+	−	+
c. *lacI*$^{+}$ *lacP*$^{-}$ *lacO*$^{+}$ *lacZ*$^{+}$ *lacY*$^{-}$	−	−	−	−
d. *lacI*$^{+}$ *lacP*$^{+}$ *lacO*$^{+}$ *lacZ*$^{-}$ *lacY*$^{-}$/*lacI*$^{-}$ *lacP*$^{+}$ *lacO*$^{+}$ *lacZ*$^{+}$ *lacY*$^{+}$	−	−	+	+

a. All the genes possess normal sequences, so the *lac* operon functions normally: when lactose is absent, the repressor protein binds to the operator and inhibits the transcription of the structural genes, so *β*-galactosidase and permease are not produced. When lactose is present, some of it is converted into allolactose, which binds to the repressor and makes it inactive; thus, the repressor does not bind to the operator, so the structural genes are transcribed and *β*-galactosidase and permease are produced.
b. The structural *lacZ* gene is mutated, so *β*-galactosidase is not produced under any conditions. The *lacO* gene has a constitutive mutation, which means that the

repressor is unable to bind to *lacO*, so transcription takes place at all times. Therefore, permease is produced in both the presence and the absence of lactose.

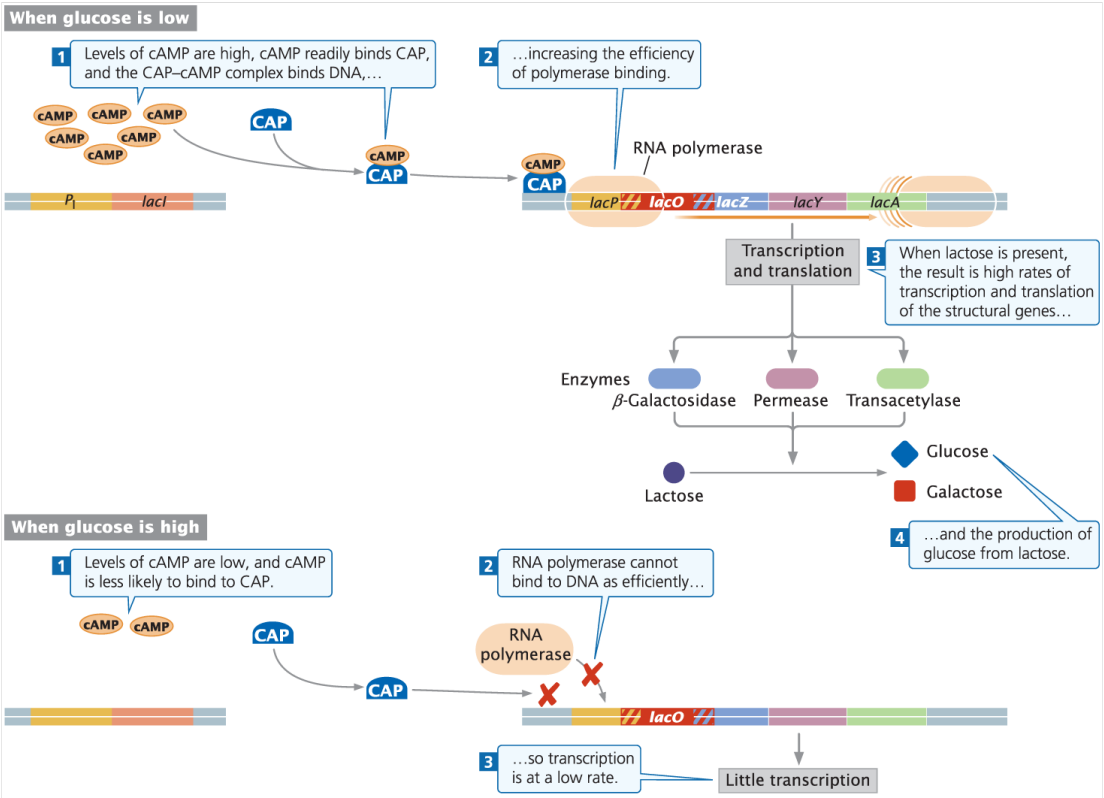
c. In this strain, the promoter is mutated, so RNA polymerase is unable to bind to it, and transcription does not take place. Therefore, *β*-galactosidase and permease are not produced under any conditions.
d. This strain is a partial diploid, which possesses two copies of the *lac* operon—one on the bacterial chromosome and the other on a plasmid. The copy of the *lac* operon represented in the upper part of the genotype (before the slash) has mutations in both the *lacZ* and the *lacY* genes, so it is not capable of encoding *β*-galactosidase or permease under any conditions. The copy represented in the lower part of the genotype (after the slash) has a defective regulator gene, but the normal regulator gene in the upper copy produces a repressor protein that is capable of diffusing to other molecules (trans acting), so in the absence of lactose, it binds to the lower copy and inhibits transcription. Therefore, no *β*-galactosidase or permease is produced when lactose is absent. In the presence of lactose, the repressor cannot bind to the operator, so the structural genes in the lower copy of the *lac* operon are transcribed, and *β*-galactosidase and permease are produced.

›› Now try your hand at predicting the outcome of different *lac* mutations by working **Problem 21** at the end of the chapter.

Positive Control and Catabolite Repression

As we've seen, *E. coli* and many other bacteria metabolize glucose preferentially, even in the presence of lactose and other sugars. They do so because glucose enters glycolysis without further modification and therefore requires less energy to metabolize than do other sugars. When glucose is available, genes that participate in the metabolism of other sugars are turned off through a process known as **catabolite repression**. Efficient transcription of the *lac* operon, for example, takes place only if lactose is present and glucose is absent. But how is the expression of the *lac* operon influenced by glucose? What brings about catabolite repression?

In spite of being termed “repression,” which might suggest negative control, catabolite repression actually results from positive control (transcription is turned on) in response to low glucose. (This regulation is in addition to the negative control brought about by a repressor binding to the operator of the *lac* operon when lactose is absent.) This positive control is accomplished through the binding of a protein called the **catabolite activator protein (CAP)** to a site that is about 22 nucleotides long and is located within or slightly upstream of the promoter of the *lac* genes (**Figure 16.12**). RNA polymerase does not bind efficiently to many promoters unless CAP is first bound to the DNA. Before CAP can bind, however, it must form a complex with a modified nucleotide called **3′, 5′-cyclic adenosine monophosphate** (cyclic AMP, or **cAMP**), which is important in cellular signaling processes in both bacterial and eukaryotic cells. In *E. coli*, the concentration of cAMP is regulated so that it is inversely proportional to the level of available glucose. High concentrations of glucose within the cell lower the amount of cAMP, so little cAMP–CAP complex is available to bind to the DNA. Consequently, RNA polymerase has poor affinity for the *lac* promoter, and little transcription of the *lac* structural genes takes place. Low concentrations of glucose stimulate high levels of cAMP, resulting in increased cAMP–CAP binding to DNA. This increase enhances the binding of RNA polymerase to the *lac* promoter and increases transcription of the *lac* genes approximately 50-fold.



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16.12 The catabolite activator protein (CAP) binds to the promoter of the *lac* operon and stimulates transcription. CAP must form a complex with 3′, 5′-cyclic adenosine monophosphate (cAMP) before binding to the promoter. The binding of cAMP–CAP to the promoter activates transcription by facilitating the binding of RNA polymerase. Levels of cAMP are inversely related to glucose levels: low concentrations of glucose stimulate high concentrations of cAMP, and high concentrations of glucose stimulate low concentrations of cAMP.

Description

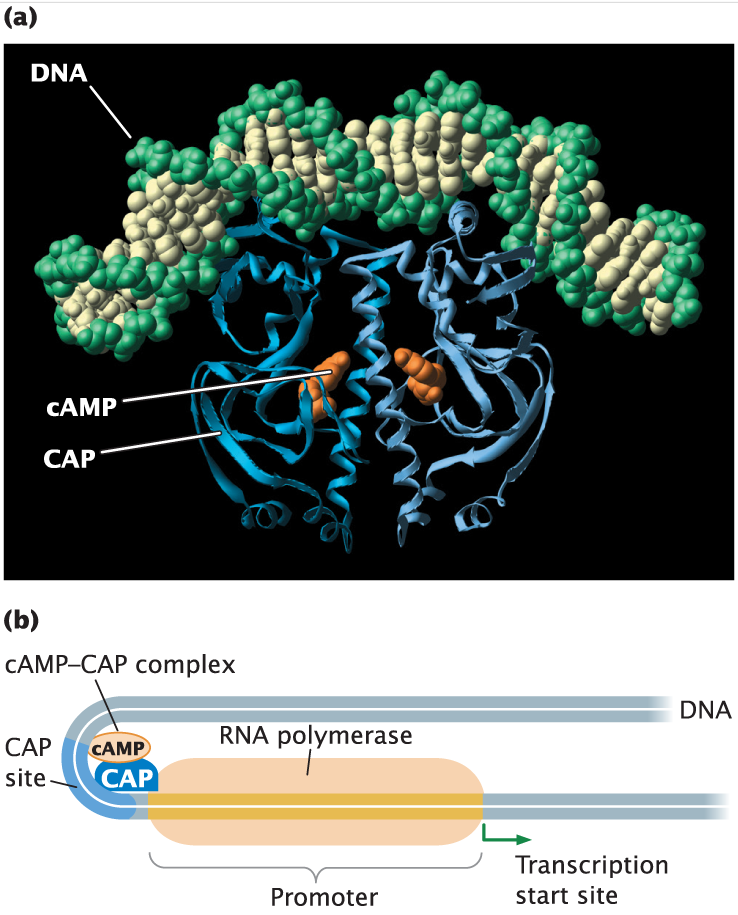
Text at the top left reads, when glucose is low. A double strand, at the top left, shows P subscript I and lac big I gene. Six c A M P molecules are present over this double strand. One C A P molecule binds to one of the c A M P molecule. A caption reads, Levels of c A M P are high, c A M P readily binds C A P, and the C A P–c A M P complex binds D N A. An operon is present on the top right and has the following components from left to right: lac big P, lac big O, lac big Z, lac big Y, lac big A. The C A P-c A M P complex attaches to the left side of the operon. An R N A polymerase binds to the lac big P and lac big O genes and move from left to right. A caption reads, increasing the efficiency of polymerase binding. Transcription and translation occurs below. A caption reads, When lactose is present, the result is high rates of transcription and translation of the structural genes. Three enzymes are formed below: b-Galactosidase corresponding to lac big Z, permease corresponding to lac big Y, and Transacetylase corresponding to lac big A. Lactose below converts to Glucose and Galactose, in the presence of the enzymes produced above. A caption reads, and the production of glucose from lactose.

Text below reads, when glucose is high. A double strand at the bottom left shows P subscript I and lac big Il gene. Two c A M P molecules are present over this double strand. A caption reads, Levels of c A M P are low, and c A M P is less likely to bind to C A P. An operon is present on the bottom right and has the following components from left to

right: lac big P, lac big O, lac big Z, lac big Y, lac big A. C A P and R N A polymerase are unable to bind to the operon. A caption reads, R N A polymerase cannot bind to D N A as efficiently, so transcription is at a low rate. Only little transcription occurs below.

The catabolite activator protein exerts positive control on more than 20 operons of *E. coli*. The response to CAP varies among these operons: some are activated by low levels of CAP, whereas others require higher levels. CAP contains a helix-turn-helix DNA-binding motif (see Figure 16.2a), and when it binds at the CAP site on DNA, it causes the DNA helix to bend (**Figure 16.13**). The bent helix enables CAP to facilitate the binding of RNA polymerase at the promoter and the initiation of transcription. **› TRY PROBLEM 15**

THINK-PAIR-SHARE Question 5



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16.13 The binding of the cAMP–CAP complex to DNA produces a sharp bend in DNA that activates transcription.

Description
The molecular model labeled a at the top shows D N A double helix in balls and stick formation. The D N A helix is bending around the c A M P–C A P complex which is represented by ribbon diagram.

The illustration b below shows a D N A double strand emerging from the right, bending on the left side and again straightening up to reach the right side. The area where the D N A is bent is labeled C A P site and it shows the c A M P–C A P complex attached to it. R N A polymerase is bound to the right of the c A M P–C A P complex. Text below reads, promoter. Transcription site is labeled to the right of promoter on the D N A strand at the bottom.

CONCEPTS

In spite of its name, catabolite repression is a type of positive control. The catabolite activator protein (CAP), complexed with cAMP, binds to a site at or near the *lac* promoter and stimulates the binding of RNA polymerase. Cellular levels of cAMP are controlled by glucose; a low glucose level increases the abundance of cAMP and enhances the transcription of the *lac* structural genes.

✔ CONCEPT CHECK 7

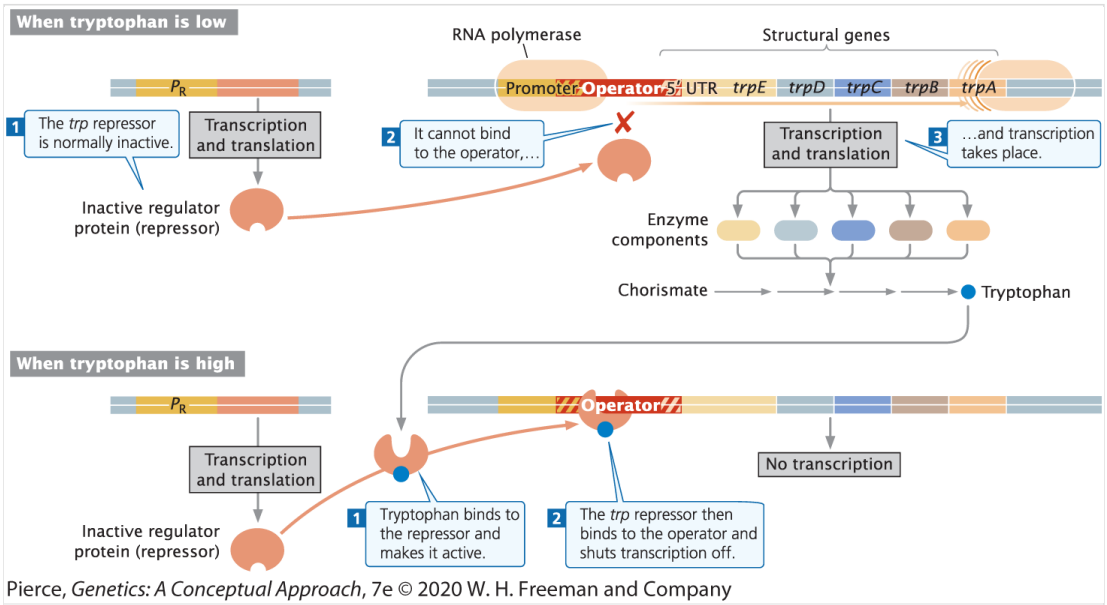
What is the effect of high levels of glucose on the *lac* operon?

a. Transcription is stimulated.
b. Little transcription takes place.
c. Transcription is not affected.
d. Transcription may be stimulated or inhibited, depending on the levels of lactose.

The *trp* Operon of *E. coli*

The *lac* operon just discussed is an inducible operon, one in which transcription does not normally take place and must be turned on. Other operons are repressible: transcription in these operons is normally turned on and must be repressed when the products of its structural genes are not needed. The tryptophan (*trp*) operon in *E. coli,* which controls the biosynthesis of the amino acid tryptophan, is an example of a negative repressible operon.

The *trp* operon contains five structural genes (*trpE*, *trpD*, *trpC*, *trpB*, and *trpA*) that produce the components of three enzymes (two of the enzymes consist of two polypeptide chains). These enzymes convert a precursor called chorismate into tryptophan (Figure 16.14). The first structural gene, *trpE*, contains a long 5′ untranslated region (5′ UTR) that is transcribed but does not encode any of these polypeptides. Instead, this 5′ UTR plays an important role in another regulatory mechanism, which we'll describe in Section 16.3. Upstream of the 5′ UTR is the *trp* promoter. When tryptophan levels are low, RNA polymerase binds to the promoter and transcribes the five structural genes into a single mRNA, which is then translated into the enzymes that convert chorismate into tryptophan.



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16.14 The *trp* operon controls the biosynthesis of the amino acid tryptophan in *E. coli*.

Description

The illustration shows Text at the top left that reads, When tryptophan is low. A double strand, at the top left, shows P subscript R and an another gene. It leads to formation of an inactive regulator protein (repressor) below, after transcription and translation. A caption reads, The t r p repressor is normally inactive. An operon is present to the right and has the following from left to right: promoter, operator, 5 prime U T R, t r p big E, t r p big D, t r p big C, t r p big B, t r p big A. The inactive repressor cannot bind to the operator. A caption reads, It cannot bind to the operator. The R N A polymerase binds to the operator and moves from left to right. Transcription and translation occurs. A caption reads, and transcription takes place. The corresponding enzyme components are formed for each gene. Chorismate converts to Tryptophan in the presence of the enzyme components synthesized above.

Text below reads, When tryptophan is high.

A double strand, at the bottom left, shows P subscript R and an another gene. It leads to formation of an inactive regulator protein (repressor) below, after transcription and translation. An operon is present to the right and has the following from left to right: promoter, operator, 5 prime U T R, t r p big E, t r p big D, t r p big C, t r p big B, t r p big AA. The inactive repressor binds to the tryptophan produced above and becomes active. A caption reads, Tryptophan binds to the repressor and makes it active. This active t r p repressor now binds to the operator. A caption reads, The t r p repressor then binds to the operator and shuts transcription off. No transcription takes place.

Some distance from the *trp* operon is a regulator gene, *trpR*, which encodes a repressor protein that is normally inactive (see Figure 16.14). Like the *lac* repressor, the tryptophan repressor has two binding sites, one that binds to the operator and another that binds to tryptophan (the corepressor). Binding with tryptophan causes a conformational change in the repressor that makes it capable of binding to the operator, which overlaps the promoter. When the operator is occupied by the repressor, RNA polymerase cannot bind to the promoter, and the structural genes cannot be transcribed. Thus, when cellular levels of tryptophan are low, transcription of the *trp* operon takes place and more tryptophan is synthesized; when cellular levels of tryptophan are high, transcription of the *trp* operon is inhibited and the synthesis of more tryptophan does not take place.

CONCEPTS

The *trp* operon is a negative repressible operon that controls the biosynthesis of tryptophan. In a repressible operon, transcription is normally turned on and must be repressed; in the case of the *trp* operon, this is accomplished through the binding of tryptophan to the repressor, which renders the repressor active. The active repressor then binds to the operator and prevents RNA polymerase from transcribing the structural genes.

✔ CONCEPT CHECK 8

In the *trp* operon, what happens to the *trp* repressor in the absence of tryptophan?

a. It binds to the operator and represses transcription.
b. It cannot bind to the operator, and transcription takes place.
c. It binds to the regulator gene and represses transcription.
d. It cannot bind to the regulator gene, and transcription takes place.

16.3 Some Operons Regulate Transcription Through Attenuation, the Premature Termination of Transcription

We've now seen several different ways in which a cell regulates the initiation of transcription in an operon. Some operons have an additional level of control that affects the *continuation* of transcription rather than its initiation. In **attenuation**, transcription begins at the transcription start site, but termination takes place prematurely, before the RNA polymerase even reaches the structural genes. Attenuation takes place in a number of operons that encode enzymes participating in the biosynthesis of amino acids.

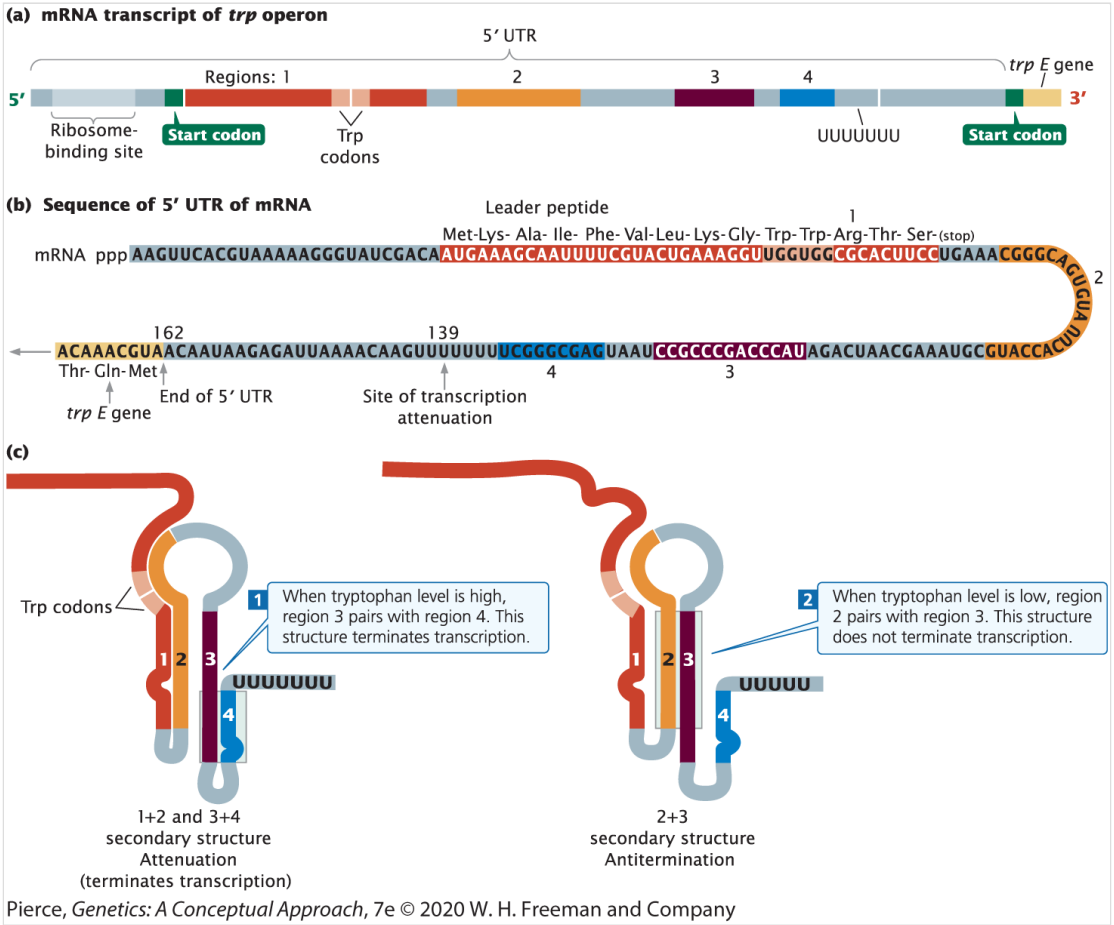
Attenuation in the *trp* Operon of *E. coli*

We can understand the process of attenuation most easily by looking at one of the best-studied examples, which is found in the *trp* operon of *E. coli*. The *trp* operon is unusual in that it is regulated both by repression and by attenuation. Most operons are regulated by one of these mechanisms but not by both of them.

Attenuation first came to light when Charles Yanofsky and his colleagues made several observations in the early 1970s that indicated that repression at the operator is not the only method of regulation of the *trp* operon. They isolated a series of mutant *E. coli* strains that exhibited high levels of transcription but in which control at the operator was unaffected, suggesting that some mechanism other than repression at the operator was controlling transcription. Furthermore, they observed that two mRNAs of different sizes were transcribed from the *trp* operon: a long mRNA containing sequences encoded by the structural genes and a much shorter mRNA of only 140 nucleotides. These observations led Yanofsky to propose that a mechanism that causes premature termination of transcription also regulates transcription in the *trp* operon.

Close examination of the *trp* operon reveals a region of 162 nucleotides that corresponds to the long 5′ UTR of the mRNA (mentioned in Section 16.2) transcribed from the *trp* operon (**Figure 16.15a**). The 5′ UTR (also called a leader) contains four regions with the following complementarities: region 1 is complementary to region 2, region 2 is complementary to

region 3, and region 3 is complementary to region 4 (Figure 16.15b). These complementarities allow the 5′ UTR to fold into two different secondary structures (Figure 16.15c). Only one of these secondary structures causes attenuation.



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16.15 Two different secondary structures can be formed by the 5′ UTR of the mRNA transcript of the *trp* operon.

Description

Part a shows m R N A transcript of t r p operon. A single strand runs from 5 prime to 3 prime. The following regions are from left to right: start of 5 prime U T R, ribosome binding site, start codon, region 1, two t r p codons, region 2, region 3, region 4, a stretch of U nucleotides, start codon, end of 5 prime U T R and t r p E gene.

Part b below shows sequence of 5 prime U T R of m R N A. The sequence of m R N A is as follows: A, G, U, U, C, A, C, G, U, A, A, A, A, A, G, G, G, U, A, U, C, G, A, C, A, leader peptide begins, A, U, G (Met), A,A,A (Lys), G, C,A (Ala), A ,U, U (Ile), U,U, C (Phe), G,U,A (Val), C, U, G (Leu) A, A, A (lys) G, G, U (Gly) U, G, G (Trp)U, G, G (Trp)C, G, C (Arg)A, C, U (Thr) U, C, C (Ser) stop, U,G, A, A, A, region 2 begins C, G, G, G, C, A, G, U, G, U, A. The remaining sequence from right to left is as follows: G, U, A, C, C, A, C, U, U (region 2 ends), A, G, A, C, U, A, A, C, G, A, A, A, U, G, C, C, C, G, C, C, C, G, A, C, C, C, A, U (region 3), U, A, A, U, U, C, G, G, G, C, G,A, G (region 4), 162 end of 5 prime U T R, A, C, A, A, U, A, A, G, A, G, A, U, U, A, A, A, C, A, A, G, U, U, U, U, U, U, U (Site of transcription Attenuation), A, C, A (thr)A, A, C (gln)G, U, A (met) (T r p big E gene),

Part c below shows two different secondary structures of m R N A. The structure of the m-R N A when the tryptophan level is high is similar to its structure when the level is low. The structure of the molecule when tryptophan is high at the left, involves regions 1 and 2 binding and regions 3 and 4 binding closely and forming stems. A stem loop is also present between regions 2 and 3 but the sides are loosely associated. A poly U tail extends after the end of region 4. Two t rp codons are adjacent to region 1. This structure terminates transcription. A caption reads, When tryptophan level is high, region 3 pairs with region 4. This structure terminates transcription. Text below reads, 1 plus 2 and 3 plus 4secondary structure Attenuation (terminates transcription).

The structure of the molecule when tryptophan is low on the right, involves regions 1 and 2 widening apart and dissociating and regions 2 and 3 forming a tight stem loop structure. Region 3 also dissociates from region 4. This structure does not terminate transcription. A caption reads, When tryptophan level is low, region 2 pairs with region 3. This structure does not terminate transcription. Text below reads, 2 plus 3 secondary structure Antitermination.

One of the secondary structures that can form in the 5′ UTR contains one hairpin produced by the base pairing of regions 1 and 2 and another hairpin produced by the base pairing of regions 3 and 4 (see Figure 16.15c, left). Notice that a string of uracil nucleotides follows the 3 + 4 hairpin. Not coincidentally, the structure of a bacterial intrinsic (rho-independent) terminator (see Figure 13.14) includes a hairpin followed by a string of uracil nucleotides; this secondary structure in the 5′ UTR of the *trp* operon is indeed a terminator of transcription and is called an **attenuator**. The attenuator forms when cellular levels of tryptophan are high, causing transcription to be terminated before the *trp* structural genes can be transcribed.

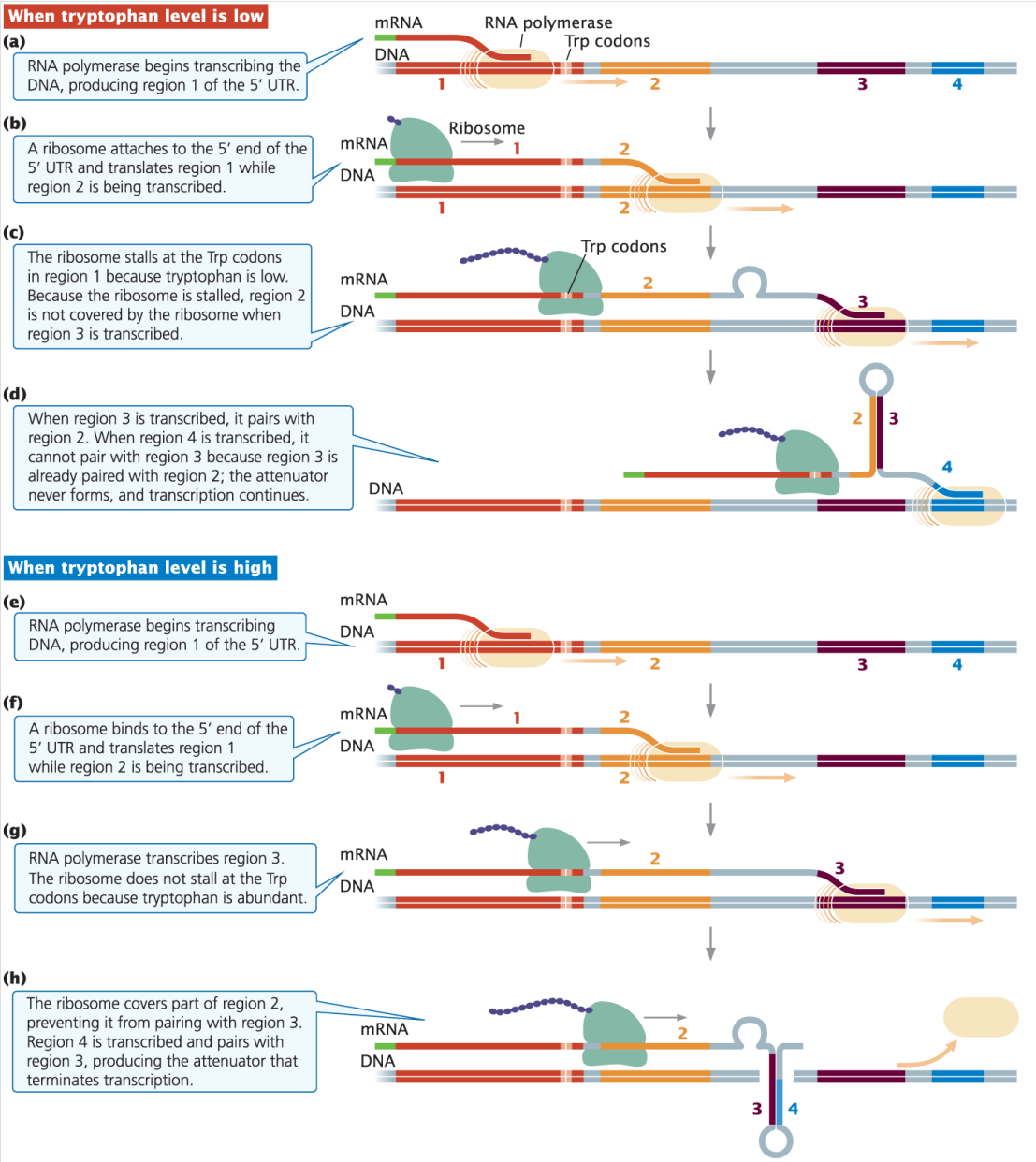
When cellular levels of tryptophan are low, however, the alternative secondary structure of the 5′ UTR is produced by the base pairing of regions 2 and 3 (see Figure 16.15c, right). This base pairing also produces a hairpin, but this hairpin is not followed immediately by a string of uracil nucleotides, so this structure does *not* function as a terminator. RNA polymerase continues past the 5′ UTR into the coding regions of the structural genes, and the enzymes that synthesize tryptophan are produced. Because it prevents the termination of transcription, the 2 + 3 structure is called an **antiterminator**.

To summarize, the 5′ UTR of the *trp* operon can fold into one of two secondary structures. When the tryptophan level is high, the 3 + 4 structure forms, transcription is terminated within the 5′ UTR, and no additional tryptophan is synthesized. When the tryptophan level is low, the 2 + 3 structure forms, transcription continues through the structural genes, and tryptophan is synthesized. The critical question, then, is this: Why does the 3 + 4 structure arise when the level of tryptophan in the cell is high, whereas the 2 + 3 structure arises when the level is low?

To answer this question, we must take a closer look at the nucleotide sequence of the 5′ UTR. At the 5′ end, upstream of region 1, is a ribosome-binding site (see Figure 16.15a). Region 1 encodes a small polypeptide. Within the coding sequence for this polypeptide are two UGG codons, which specify the amino acid tryptophan, so tryptophan is required for the translation of this 5′ UTR sequence. The small polypeptide encoded by this part of the 5′ UTR is presumed to be unstable; its only apparent function is to control attenuation. Although it was stated in Chapter 14 that 5′ UTR are not translated into proteins, the 5′ UTR of operons subject to attenuation are exceptions to this rule. The precise timing and interaction of transcription and translation in the 5′ UTR determine whether attenuation takes place.

TRANSCRIPTION WHEN TRYPTOPHAN LEVELS ARE LOW

Let's first consider what happens when intracellular levels of tryptophan are low. Recall that in prokaryotic cells, transcription and translation are coupled: while transcription is taking place at the 3′ end of the mRNA, translation is initiated at the 5′ end. RNA polymerase begins transcribing the DNA, producing region 1 of the 5′ UTR (**Figure 16.16a**). Closely following RNA polymerase, a ribosome binds to the 5′ UTR and begins to translate the protein-coding region. Meanwhile, RNA polymerase is transcribing region 2 (**Figure 16.16b**). Region 2 is complementary to region 1, but because the ribosome is translating region 1, the nucleotides in regions 1 and 2 cannot base pair.



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16.16 Whether the premature termination of transcription (attenuation) takes place in the *trp* operon depends on the cellular level of tryptophan.

Description

Text at the top reads, When tryptophan level is low. Part a below shows a double stranded D N A, with the following regions from left to right: region 1, t r p codons, region 2, region 3, region 4. An R N A polymerase is bound at region 1 and an m R N A extends out form the R N A polymerase. A caption reads, R N A polymerase begins transcribing the D N A, producing region 1 of the 5 prime U T R.

Part b below shows R N A polymerase has moved to the region 2 and a ribosome is attached to the now elongated m R N A. A caption reads, A ribosome attaches to the 5 prime end of the 5 prime U T R and translates region 1 while region 2 is

being transcribed.

Part c below shows ribosome has now moved to the right on the m R N A strand and is above region 2.A stem loop is formed before region 3. The R N A polymerase is at region 3 now. A small chain of circular structures is extending out of ribosome. A caption reads, The ribosome stalls at the T r p codons in region 1 because tryptophan is low. Because the ribosome is stalled, region 2 is not covered by the ribosome when region 3 is transcribed.

Part d below shows R N A polymerase has now moved to region 4 and the part 2 and 3 of the m R N A synthesized is almost joined and forming a loop overhead. A caption reads, When region 3 is transcribed, it pairs with region 2. When region 4 is transcribed, it cannot pair with region 3 because region 3 is already paired with region 2; the attenuator never forms, and transcription continues.

Text below reads, when the tryptophan level is high.

Part e below shows a double stranded D N A, with the following regions from left to right: region 1, t r p codons, region 2, region 3, region 4. An R N A polymerase is bound at region 1 and an m R N A extends out form the R N A polymerase. A caption reads, R N A polymerase begins transcribing the D N A, producing region 1 of the 5 prime U T R.

Part f below shows R N A polymerase has moved to the region 2 and a ribosome is attached to the now elongated m R N A. A caption reads, A ribosome binds to the 5 prime end of the 5 prime U T R and translates region 1 while region 2 is being transcribed.

Part g shows ribosome has now moved to the right on the m R N A strand and is just before region 2. The R N A polymerase is at region 3 now. A small chain of circular structures is extending out of ribosome. A caption reads, R N A polymerase transcribes region 3. The ribosome does not stall at the T r p codons because tryptophan is abundant.

Part h below shows the R N A polymerase has dissociated from the unit. Region 3 and 4 on the m R N A are closely associated and forming a loop below the D N A strand. The ribosome is now on region 2. A caption reads, The ribosome covers part of region 2, preventing it from pairing with region 3. Region 4 is transcribed and pairs with region 3, producing the attenuator that terminates transcription.

RNA polymerase begins to transcribe region 3, and the ribosome reaches the Trp codons (UGG) in region 1. When it reaches the Trp codons, the ribosome stalls (**Figure 16.16c**) because the level of tryptophan is low and tRNAs charged with tryptophan are scarce or unavailable. The ribosome sits at the Trp codons, awaiting the arrival of a tRNA charged with tryptophan. Stalling of the ribosome does not, however, hinder transcription; RNA polymerase continues to move along the DNA, and transcription gets ahead of translation.

Because the ribosome is stalled at the Trp codons in region 1, region 2 is free to base pair with region 3, forming the 2 + 3 hairpin (**Figure 16.16d**). This hairpin does not cause termination, so transcription continues. Because region 3 is already paired with region 2, the 3 + 4 hairpin (the attenuator) never forms, so attenuation does not take place, and transcription continues. RNA polymerase continues along the DNA, past the 5′ UTR, transcribing all the structural genes into mRNA, which is translated into the enzymes encoded by the *trp* operon. These enzymes then synthesize tryptophan.

TRANSCRIPTION WHEN TRYPTOPHAN LEVELS ARE HIGH

Now let's see what happens when intracellular levels of tryptophan are high. Once again, RNA polymerase begins transcribing the DNA, producing region 1 of the 5′ UTR (**Figure 16.16e**). Closely following RNA polymerase, a ribosome binds to the 5′ UTR and begins to translate the protein-coding region (**Figure 16.16f**). When the ribosome reaches the two Trp codons, it doesn't slow or stall because tryptophan is abundant and tRNAs charged with tryptophan are readily available (**Figure 16.16g**). This point is critical to note: because tryptophan is abundant, translation can keep up with transcription.

As it moves past region 1, the ribosome partly covers region 2 (**Figure 16.16h**); meanwhile, RNA polymerase completes the transcription of region 3. Although regions 2 and 3 are complementary, the ribosome physically blocks their pairing.

RNA polymerase continues to move along the DNA, eventually transcribing region 4 of the 5′ UTR. Region 4 is complementary to region 3, and because region 3 cannot base pair with region 2, it pairs with region 4. The pairing of regions 3 and 4 (see Figure 16.16h) produces the attenuator, and transcription terminates just beyond region 4. The structural genes are not transcribed, no tryptophan-producing enzymes are translated, and no additional tryptophan is synthesized.

Important events in the process of attenuation are summarized in **Table 16.3**. Try pausing the ribosome at the Trp codons for different lengths of time in (A)**Animation 16.2** and see what effect the pause has on transcription.

TABLE 16.3 Events in the process of attenuation

Intracellular Level of Tryptophan	Ribosome Stalls at Trp Codons	Position of Ribosome When Region 3 Is Transcribed	Secondary Structure of 5′ UTR	Termination of Transcription of *trp* Operon
High	No	Covers region 2	3 + 4 hairpin	Yes
Low	Yes	Covers	2 + 3 hairpin	No

region 1

A key factor controlling attenuation is the number of tRNA molecules charged with tryptophan because their availability is what determines whether the ribosome stalls at the Trp codons. A second factor is the synchronization of transcription and translation, which is critical to attenuation. Synchronization is achieved through a site located in region 1 of the 5′ UTR where RNA polymerase pauses. When this site is transcribed, the RNA folds into a secondary structure that inhibits further transcription. Thus, the RNA polymerase stops temporarily at the pause site, allowing time for a ribosome to bind to the 5′ end of the mRNA. As the ribosome approaches the secondary structure in the RNA, it disrupts that structure and allows transcription to continue. Translation then closely follows transcription. It is important to point out that ribosomes do not traverse the convoluted hairpins of the 5′ UTR to translate the structural genes. Ribosomes that attach to the 5′ end of region 1 of the mRNA encounter a stop codon at the end of region 1. New ribosomes translating the structural genes attach to a different ribosome-binding site located near the beginning of the *trpE* gene (see Figure 16.15a). ❯ **TRY PROBLEM 29**

THINK-PAIR-SHARE Questions 6 and 7

Why Does Attenuation Take Place in the *trp* Operon?

Why do bacteria need attenuation in the *trp* operon? Shouldn't repression at the operator prevent transcription from taking place when tryptophan levels in the cell are high? Why does the cell have two types of control? Part of the answer is that repression is never complete. Some transcription is initiated even when the *trp* repressor is active; repression reduces transcription only as much as 70-fold. Attenuation can further reduce transcription another 8- to 10-fold, so together, the two processes are capable of reducing transcription of the *trp* operon more than 600-fold. Both mechanisms provide *E. coli* with a much finer degree of control over tryptophan synthesis than either could achieve alone.

Another reason for this dual control is that attenuation and repression respond to different signals: repression responds to cellular levels of tryptophan, whereas attenuation responds to the availability of tRNAs charged with tryptophan. There might be circumstances in which a cell's ability to respond to these different signals is advantageous. Finally, the *trp*

repressor affects several operons in addition to the *trp* operon. At an early stage in the evolution of *E. coli*, the *trp* operon may have been controlled only by attenuation. The *trp* repressor may have evolved primarily to control the other operons and only incidentally affects the *trp* operon.

Attenuation is a difficult process to grasp because you must simultaneously visualize two dynamic processes—transcription and translation—and it's easy to confuse them. Remember that attenuation refers to the early termination of *transcription*, not translation (although events in translation bring about the termination of transcription). Attenuation often causes confusion because we know that transcription must precede translation. We're comfortable with the idea that transcription might affect translation, but it's harder to imagine that the effects of translation could influence transcription, as they do in attenuation. The reality is that transcription and translation are closely coupled in prokaryotic cells, and events in one process can easily affect the other.

CONCEPTS

The *trp* operon regulates transcription through attenuation as well as through repression. In attenuation, transcription is initiated but is terminated prematurely. When tryptophan levels are low, the ribosome stalls at Trp codons, and transcription continues. When tryptophan levels are high, the ribosome does not stall at Trp codons, and the 5′ UTR adopts a secondary structure that terminates transcription before the structural genes can be transcribed.

✔ CONCEPT CHECK 9

Attenuation results when which regions of the 5′ UTR pair?

a. 1 and 3
b. 2 and 3
c. 2 and 4
d. 3 and 4

16.4 Other Sequences Control the Expression of Some Bacterial Genes

Several types of sequences outside operons affect the expression of some genes in bacteria. These sequences include bacterial enhancers and several types of RNA regulators.

Bacterial Enhancers

An enhancer is a DNA element that affects transcription, but in contrast to promoters, an enhancer is typically found some distance from the gene whose transcription it affects (see Chapter 17). Enhancers were originally described in eukaryotes, but research now indicates that some also occur in bacteria and archaea.

Like enhancers in eukaryotes, bacterial enhancers contain binding sites for proteins that increase the rate of transcription at promoters that are distant from the enhancer. They do this by causing the DNA between the promoter and the enhancer to loop out, so that the transcription activator bound to the enhancer directly interacts with RNA polymerase at the promoter. Enhancers are position independent, meaning that they can be moved without affecting their ability to enhance transcription. Most bacterial enhancers are found upstream of genes that use a special type of sigma factor (see

Chapter 13) known as sigma 54 (σ^{54}). Enhancers will be discussed in more detail in Chapter 17.

CONCEPTS

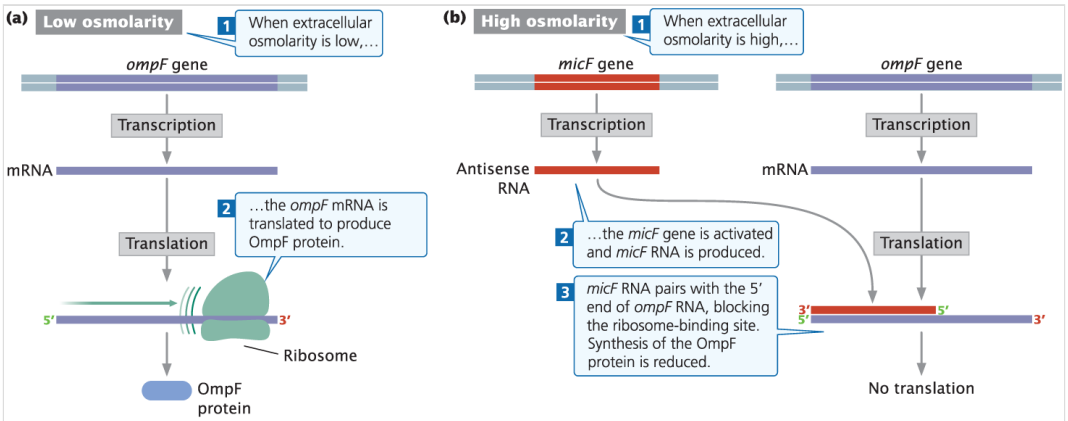
Bacterial enhancers increase the rate of transcription at genes that are distant from the enhancer.

Antisense RNA

Some RNA molecules are complementary to particular sequences on mRNAs. These molecules, called **antisense RNA**, control gene expression by binding to sequences on mRNA and inhibiting its translation. Translational control by antisense RNA is seen in the regulation of the *ompF* gene of *E. coli* (**Figure 16.17a**). This gene encodes an outer-membrane protein that functions as a channel for the passive diffusion of small polar molecules, such as water and ions, across the membrane. Under most conditions, the *ompF* gene is transcribed and translated and the OmpF protein is synthesized. However, when the osmolarity of the medium increases, the cell depresses the production of OmpF protein to help maintain cellular osmolarity. A regulatory gene named *micF*—for *m*RNA-*i*nterfering *c*omplementary RNA—is activated, and *micF* RNA is produced (**Figure 16.17b**). The *micF* RNA, an antisense RNA, binds to a complementary sequence in the 5′ UTR of the *ompF* mRNA and

inhibits the binding of the ribosome. This inhibition reduces the amount of translation (see Figure 16.17b), which results in fewer OmpF proteins in the outer membrane and thus reduces the movement of substances across the membrane owing to the changes in osmolarity. Research has detected many antisense RNA molecules in a wide range of bacterial species. **› TRY PROBLEM 32**

THINK-PAIR-SHARE Question 8



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16.17 Antisense RNA can regulate translation.

Description

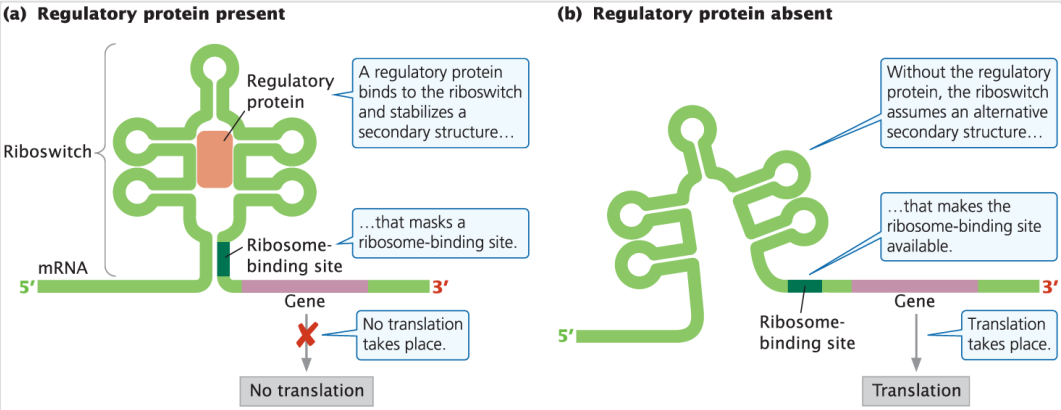
Part a labeled low osmolarity on the left shows a double strand with small O small M small P big F gene in the center. A caption reads, When extracellular osmolarity is low. Transcription occurs and a single stranded m R N A is formed below. Translation occurs and a ribosome moves from left to right on the m R N A formed. A caption reads, the small O small M small P big F m R N A is translated to produce small O small M small P big F protein. A small O small M small P big F protein is obtained below.

Part b labeled High osmolarity, on the right, shows a double strand with gene small M small I small C big F gene in the center. Transcription occurs and a single stranded m R N A is obtained below. A caption reads, When extracellular osmolarity is high, the small M small I

small C big F gene is activated and small M small I small C big F R N A is produced. A double strand with gene small O small M small P big F gene in the center is present on the right side of the double strand with gene small M small I small C big F gene. Transcription occurs and a single stranded m R N A is formed below. Translation occurs and the anti sense m R N A formed on the left is placed over the m R N A formed above. No translation occurs further. A caption reads, small M small I small C big F R N A pairs with the 5 prime end of small O small M small P big F R N A, blocking the ribosome-binding site.

Riboswitches

We have seen that operons of bacteria contain DNA sequences (promoters and operator sites) where the binding of small molecules induces or represses transcription. Some mRNA molecules contain regulatory sequences called **riboswitches**, where regulatory molecules can bind and affect gene expression by influencing the formation of secondary structures in the mRNA (**Figure 16.18**). Riboswitches appear to be common in bacteria, regulating about 4% of all bacterial genes. They are also present in archaea, fungi, and plants.



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16.18 Riboswitches are RNA sequences in mRNA that affect gene expression.

Description

Part a on the left, labeled regulatory protein present, shows the secondary m-R N A structure. The structure shows a loop labeled riboswitch, which has 2 stem loops projecting on the right, 1 stem loop projecting at the top, and 2 stem loops projecting on the left. A regulatory protein is present and binds at the center of the loops. A caption reads, A regulatory protein binds to the riboswitch and stabilizes a secondary structure. A ribosome binding site is present after the loops on the right side followed by a gene. A caption reads, that masks a ribosome-binding site. No translation of the gene takes place. A caption reads, No translation takes place.

Part b on the right, labeled Regulatory protein absent, shows the secondary m-R N A structure. The secondary structure contains a loop with 5 stem loops projecting on the same sides as in part a, but the bottom of the ribo-switch does not pinch at the other bottom. The regulator protein is absent. A caption reads, Without the regulatory protein, the riboswitch assumes an alternative secondary structure. The ribosome-binding site is accessible and a gene is present to its right. A caption reads, that makes the ribosome-binding site available. Translation of the gene takes place. A caption reads, Translation takes place.

Riboswitches, which are typically found in the 5′ UTR of the mRNA, can fold into compact RNA secondary structures with a base stem and several branching hairpins. In some cases, a small regulatory molecule binds to the riboswitch and stabilizes a terminator, which causes premature termination of transcription. In other cases, the binding of a regulatory molecule stabilizes a secondary structure that masks the ribosome-binding site, preventing the initiation of translation. When not bound by the regulatory molecule, the riboswitch assumes an alternative structure that eliminates the premature terminator or makes the ribosome-binding site available.

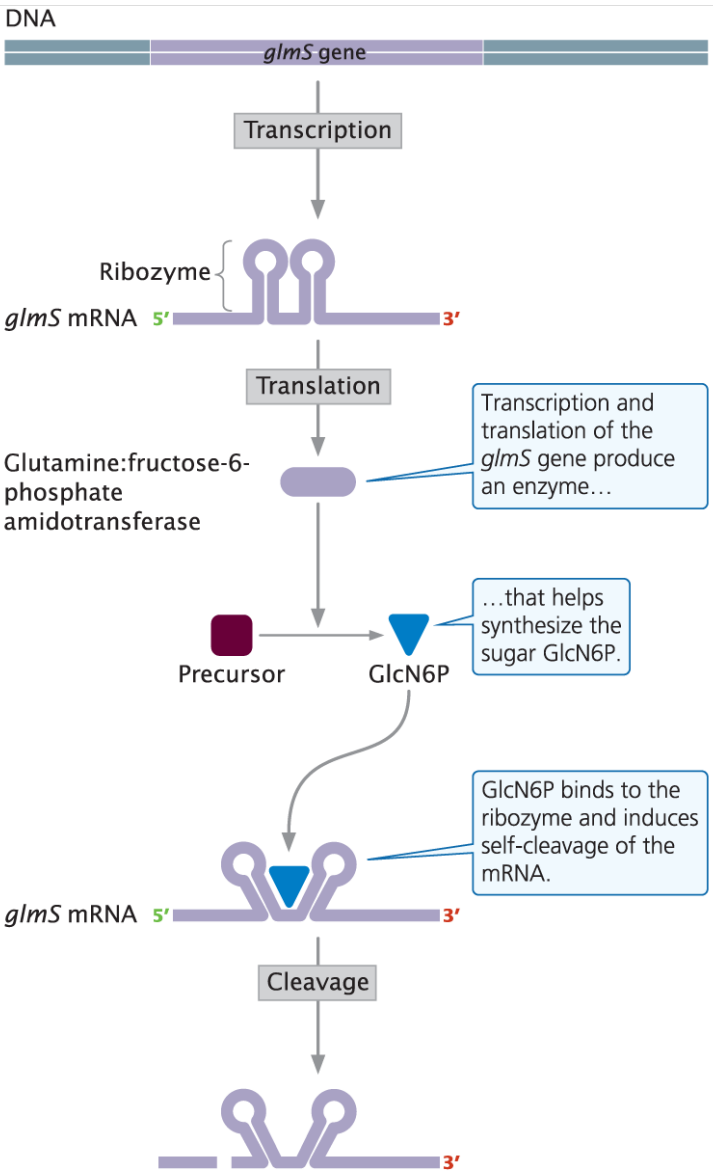
A RIBOSWITCH CONTROLS THE SYNTHESIS OF VITAMIN B12

An example of a riboswitch is seen in the bacterial genes that encode enzymes that play roles in the synthesis of vitamin B12. The genes for these enzymes are transcribed into an mRNA molecule that contains a riboswitch. When the activated form of vitamin B12—called coenzyme B12—is present, it binds to the riboswitch, and the mRNA folds into a secondary structure that obstructs the ribosome-binding site. Consequently, no translation of the mRNA takes place. In the absence of coenzyme B12, the mRNA assumes a different secondary structure. This secondary structure does not obstruct the ribosome-binding site, so translation is initiated, the enzymes are synthesized, and vitamin B12 is produced. For some riboswitches, the regulatory molecule acts as a repressor (as just described) by inhibiting transcription or translation; for others, the regulatory molecule acts as an inducer by causing the formation of a secondary structure that allows transcription or translation to take place.

RNA-Mediated Repression Through Ribozymes

Another type of gene control is carried out by mRNA molecules called ribozymes, which possess catalytic activity (see Chapter 14). Termed RNA-mediated repression, this type of control has been

demonstrated in the *glmS* gene of the bacterium *Bacillus subtilis*. Transcription of this gene produces an mRNA molecule that encodes the enzyme glutamine:-fructose-6-phosphate amidotransferase (**Figure 16.19**), which helps synthesize a small sugar called glucosamine-6-phosphate (GlcN6P). Within the 5′ UTR of the *glmS* mRNA are about 75 nucleotides that act as a ribozyme. When GlcN6P is absent from the cell, the *glmS* gene is transcribed and translated to produce the enzyme, which synthesizes GlcN6P. However, when sufficient GlcN6P is present, the sugar binds to the ribozyme sequence of the mRNA, which then induces self-cleavage of the mRNA—the ribozyme breaks the sugar–phosphate backbone of the RNA. This cleavage prevents translation of the mRNA.



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16.19 Ribozymes, when bound by small regulatory molecules, can induce the cleavage and degradation of mRNA.

Description

The illustration shows a double stranded D N A at the top with gene small G small L small M big S at the center. Translation takes place and an m R N A is obtained below. The m R N A runs from 5 prime to 3 prime and has two loops with stem at their respective tops. The loops are closely associated and are labeled ribozyme. Translation takes place and a

Glutamine: fructose-6- phosphate amidotransferase is obtained below. A caption reads, Transcription and translation of the small G small L small M big S gene produce an enzyme. A precursor below converts to a sugar labeled G l c N 6 Pm, in the presence of Glutamine: fructose-6-phosphate amidotransferase. A caption reads, that helps synthesize the sugar G l c N 6 P. The G l c N 6 P formed fits between the closely spaced ribozymes below thereby making them drift apart. A caption reads, G l c N 6 P binds to the ribozyme and induces self-cleavage of the m R N A. Cleavage occurs below and the a small single strand fragment on the 5 prime end gets separated from the ribozymes part.

CONCEPTS

In bacterial cells, antisense RNA can inhibit translation by binding to complementary sequences in the 5′ UTR of mRNA and preventing the attachment of a ribosome. Riboswitches are sequences in mRNA molecules that bind regulatory molecules and induce changes in the secondary structure of the mRNA that affect its translation. In RNA-mediated repression, a ribozyme sequence on the mRNA induces self-cleavage and degradation of the mRNA when bound by a regulatory molecule.

Chapter 16 Review

CHAPTER SUMMARY

Section 16.1 Learning Objective: Be able to explain why regulation of gene expression is important and the different levels at which gene regulation may occur.

- Gene expression can be controlled at different levels along the molecular pathway from DNA to protein, including the alteration of DNA or chromatin structure, transcription, mRNA processing, mRNA stability, translation, and posttranslational modification. Much of gene regulation takes place through the action of regulatory proteins that recognize and bind to regulatory elements in DNA.

Section 16.2 Learning Objective: Be able to explain how operons control transcription in bacterial genes.

- Genes in bacterial cells are typically clustered into operons—groups of functionally related structural genes and the sequences that control their transcription. Structural genes in an operon are transcribed together as a single mRNA molecule.
- In negative control, a repressor protein binds to DNA and inhibits transcription. In positive control, an activator protein binds to DNA and stimulates transcription. In inducible

operons, transcription is normally off and must be turned on; in repressible operons, transcription is normally on and must be turned off.

- The *lac* operon of *E. coli* is a negative inducible operon. In the absence of lactose, a repressor binds to the operator and prevents the transcription of genes that encode *β*-galactosidase, permease, and transacetylase. When lactose is present, some of it is converted into allolactose, which binds to and inactivates the repressor, allowing the structural genes to be transcribed.
- Positive control of the *lac* operon and other operons occurs through catabolite repression. When complexed with cyclic AMP, the catabolite activator protein binds to a site at or near the promoter and stimulates the transcription of the structural genes. Levels of cAMP are inversely correlated with glucose, so low levels of glucose stimulate transcription and high levels inhibit transcription.
- The *trp* operon of *E. coli* is a negative repressible operon that controls the biosynthesis of tryptophan.

Section 16.3 Learning Objective: Be able to describe the process of gene regulation through attenuation.

- Attenuation causes premature termination of transcription. It takes place through the close coupling of transcription and translation and depends on the secondary structure of the 5′ UTR sequence.

Section 16.4 Learning Objective: Be able to describe how sequences outside the operon control gene expression in bacteria.

- Bacterial enhancers increase the rate of transcription at genes that are distant from the enhancer.
- Antisense RNAs are complementary to sequences in mRNA and may inhibit translation by binding to these sequences, thereby preventing the attachment or progress of the ribosome.
- When bound by a regulatory molecule, riboswitches in an mRNA molecule induce changes in the secondary structure of the mRNA that affect its translation. Some mRNAs possess ribozyme sequences that induce self-cleavage and degradation when bound by a regulatory molecule.

WORKED PROBLEMS

Problem 1

The *fox* operon, which has sequences *A*, *B*, *C*, and *D* (which may represent either structural genes or regulatory sequences), encodes enzymes 1 and 2. These enzymes are normally produced in the presence of compound Fox. Mutations in sequences *A*, *B*, *C*, and *D* have the following effects, where a plus sign (+) indicates that the enzyme is synthesized and a minus sign (−) indicates that the enzyme is not synthesized.

	Fox absent		Fox present	
Mutation in sequence	**Enzyme 1**	**Enzyme 2**	**Enzyme 1**	**Enzyme 2**
No mutation	–	–	+	+
A	–	–	–	+
B	–	–	–	–
C	–	–	+	–
D	+	+	+	+

a. Is the *fox* operon inducible or repressible?
b. Indicate which sequence (*A*, *B*, *C*, or *D*) is part of the following components of the operon. Each sequence should be used only once.
Regulator gene _____
Promoter _____
Structural gene for enzyme 1 _____
Structural gene for enzyme 2 _____

» Solution Strategy

What information is required in your answer to the problem?

a. Whether the *fox* operon is inducible or repressible.
b. Which sequence represents each part of the operon.

What information is provided to solve the problem?

For each mutation, whether enzyme 1 and enzyme 2 are produced in the presence and absence of Fox.

For help with this problem, review:

Section 16.2.

» Solution Steps

a. When no mutations are present, enzymes 1 and 2 are produced in the presence of Fox but not in its absence, indicating that the operon is inducible and that Fox is the inducer.

b. The mutation in *A* allows the production of enzyme 2 in the presence of Fox, but enzyme 1 is not produced in the presence or absence of Fox, and so *A* must have a mutation in the structural gene for enzyme 1. With the mutation in *B*, neither enzyme is produced under any conditions, so this mutation most likely occurs in the promoter and prevents RNA polymerase from binding. The mutation in *C* affects only enzyme 2, which is not produced in the presence or absence of Fox; enzyme 1 is produced normally (only in the presence of Fox), so the mutation in *C* most likely occurs in the structural gene for enzyme 2. The mutation in *D* is constitutive, allowing the production of enzymes 1 and 2 whether or not Fox is present. This mutation most likely occurs in the regulator gene, producing a defective repressor that is unable to bind to the operator under any conditions.

Regulator gene *D*	Structural gene for enzyme 1 *A*
Promoter *B*	Structural gene for enzyme 2 *C*

Problem 2

A mutation occurs in the 5′ UTR of the *trp* operon that reduces the ability of region 2 to pair with region 3. What will the effect of this mutation be when the tryptophan level is high? When the tryptophan level is low?

» Solution Strategy

What information is required in your answer to the problem?

The effect of the mutation when tryptophan is high and when it is low.

What information is provided to solve the problem?

- The mutation occurs in the 5′ UTR of the *trp* operon.
- The mutation reduces the ability of region 2 to pair with region 3.

For help with this problem, review:

Section 16.3.

» Solution Steps

When the tryptophan level is high, regions 2 and 3 do not normally pair, and therefore the mutation will have no effect. When the tryptophan level is low, however, the ribosome normally stalls at the Trp codons in region 1 and does not cover region 2, so regions 2 and 3 are free to pair, which prevents regions 3 and 4 from pairing and forming a terminator, which would end transcription. If regions 2 and 3 cannot pair, then regions 3 and 4 will pair even when tryptophan is low, and attenuation will always take place. Therefore, no tryptophan will be synthesized even in the absence of tryptophan.

Hint: Review Figure 16.16 for a summary of attenuation.

IMPORTANT TERMS

gene regulation
structural gene
regulatory gene
constitutive
regulatory element
domain
operon
regulator gene
regulator protein
operator
negative control

positive control
inducible operon
repressible operon
inducer
allosteric protein
corepressor
coordinate induction
partial diploid
catabolite repression
catabolite activator protein (CAP)
3′, 5′-cyclic adenosine monophosphate (cAMP)
attenuation
attenuator
antiterminator
antisense RNA
riboswitch

ANSWERS TO CONCEPT CHECKS

1. A constitutive gene is not regulated and is expressed continually.
2. Transcription is the first step in the process of information transfer from DNA to protein. For cellular efficiency, gene expression is often regulated early in the process of protein production.
3. b
4. d

5. d
6. c
7. b
8. b
9. d

Chapter 16 Assessment

COMPREHENSION QUESTIONS

Section 16.1

1. Why is gene regulation important for bacterial cells?
2. Name six different levels at which gene expression might be controlled.

Section 16.2

3. Draw a picture illustrating the general structure of an operon, and identify its parts.
4. What is the difference between positive and negative control? What is the difference between inducible and repressible operons?
5. Briefly describe the *lac* operon and how it controls the metabolism of lactose.
6. What is catabolite repression? How does it allow a bacterial cell to use glucose in preference to other sugars?

Section 16.3

7. What is attenuation? What are the mechanisms by which the attenuator forms when tryptophan levels are high and the antiterminator forms when tryptophan levels are low?

Section 16.4

8. What is antisense RNA? How does it control gene expression?
9. What are riboswitches? How do they control gene expression? How do riboswitches differ from RNA-mediated repression?

APPLICATION QUESTIONS AND PROBLEMS

Section 16.1

10. CONNECT How are **Figure 16.1** and **Figure 10.17a** similar and how are they different in terms of their depictions of the flow of information within the cell?

11. Examine **Figure 16.2b**. Why do you think the motif of the DNA-binding protein shown is called a zinc-finger motif?

Section 16.2

12. *For each of the following types of transcriptional control, indicate whether the protein produced by the regulator gene will be synthesized initially as an active repressor or as an inactive repressor.
    a. Negative control in a repressible operon
    b. Negative control in an inducible operon

13. A mutation at the operator prevents the regulator protein from binding. What effect will this mutation have in the following types of operons?
    a. Regulator protein is a repressor of a repressible operon.
    b. Regulator protein is a repressor of an inducible operon.

14. The *blob* operon produces enzymes that convert compound A into compound B. The operon is controlled by regulator gene *S*. Normally, the enzymes are synthesized only in the absence of compound B. If gene *S* is mutated, the enzymes are synthesized in the presence *and* in the absence of compound B. Does gene *S* produce a regulator protein that exhibits positive or negative control? Is this operon inducible or repressible?

15. *A mutation prevents the catabolite activator protein (CAP) from binding to the promoter in the *lac* operon. What will the effect of this mutation be on the transcription of the operon?

16. CONNECT Transformation is a process in which bacteria take up new DNA released by dead cells and integrate it into their own genomes (see Chapter 9). In *Streptococcus pneumoniae* (which causes many cases of pneumonia, inner-ear infections, and meningitis), the ability to carry out transformation requires from 105 to 124 genes, collectively termed the *com* regulon. The *com* regulon is activated in response to a protein called competence-stimulating peptide (CSP), which is produced by the bacteria and exported into the surrounding medium. When enough CSP accumulates, it attaches to a receptor on the bacterial cell membrane, which then activates a regulator protein that stimulates the transcription of genes within the *com* regulon and sets in motion a series of reactions that ultimately result in transformation. Does the *com* regulon in *S. pneumoniae* exhibit positive or negative control? Explain your answer.

17. Under which of the following conditions would a *lac* operon produce the greatest amount of *β*-galactosidase? The least? Explain your reasoning.

	Lactose present	Glucose present
Condition 1	Yes	No
Condition 2	No	Yes
Condition 3	Yes	Yes
Condition 4	No	No

18. CONNECT Were the partial diploid strains of *E. coli* used by Jacob and Monod in their analysis of the *lac* operon F^+, F^-, or F' cells? Explain your answer. (See Section 9.2 for a discussion of these different types of bacterial cells.)

19. A mutant strain of *E. coli* produces *β*-galactosidase in both the presence *and* the absence of lactose. Where in the operon might the mutation in this strain be located?

20. Examine **Figure 16.7**. What would be the effect of a drug that altered the structure of allolactose so that it was unable to bind to the regulator protein?

21. *For *E. coli* strains with the following *lac* genotypes (and genotypes on the next page), use a plus sign (+) to indicate the synthesis of *β*-galactosidase and permease and a minus sign (−) to indicate no synthesis of the proteins.

	Lactose absent		Lactose present	
Genotype of strain	***β*-Galactosidase**	**Permease**	***β*-Galactosidase**	**Permease**
$lacI^+\ lacP^+\ lacO^+\ lacZ^+\ lacY^+$				
$lacI^-\ lacP^+\ lacO^+\ lacZ^+\ lacY^+$				
$lacI^+\ lacP^+\ lacO^C\ lacZ^+\ lacY^+$				
$lacI^-\ lacP^+\ lacO^+\ lacZ^+\ lacY^-$				
$lacI^-\ lacP^-\ lacO^+\ lacZ^+\ lacY^+$				
$lacI^+\ lacP^+\ lacO^+\ lacZ^-\ lacY^+/lacI^-\ lacP^+\ lacO^+\ lacZ^+\ lacY^-$				
$lacI^-\ lacP^+\ lacO^C\ lacZ^+\ lacY^+/lacI^+\ lacP^+\ lacO^+\ lacZ^-\ lacY^-$				
$lacI^-\ lacP^+\ lacO^+\ lacZ^+\ lacY^-/lacI^+\ lacP^-\ lacO^+\ lacZ^-\ lacY^+$				
$lacI^+\ lacP^-\ lacO^C\ lacZ^-\ lacY^+/lacI^-\ lacP^+\ lacO^+\ lacZ^+\ lacY^-$				

$lacI^+\ lacP^+\ lacO^+\ lacZ^+\ lacY^+/lacI^+\ lacP^+\ lacO^+\ lacZ^+\ lacY^+$	_____	_____	_____	_____
$lacI^S\ lacP^+\ lacO^+\ lacZ^+\ lacY^-/lacI^+\ lacP^+\ lacO^+\ lacZ^-\ lacY^+$	_____	_____	_____	_____
$lacI^S\ lacP^-\ lacO^+\ lacZ^-\ lacY^+/lacI^+\ lacP^+\ lacO^+\ lacZ^+\ lacY^+$	_____	_____	_____	_____

22. Give all possible genotypes of a *lac* operon that produces, or fails to produce, *β*-galactosidase and permease under the following conditions. Do not give partial-diploid genotypes.

	Lactose absent		Lactose present	
	β-Galactosidase	**Permease**	**β-Galactosidase**	**Permease**
a.	–	–	+	+
b.	–	–	–	+
c.	–	–	+	–
d.	+	+	+	+
e.	–	–	–	–
f.	+	–	+	–
g.	–	+	–	+

23. *Explain why mutations in the *lacI* gene are trans in their effects, but mutations in the *lacO* gene are cis in their effects.

24. CONNECT Which strand of DNA (upper or lower) in Figure 16.8 is the template strand? (see Chapter 13 for a discussion of the template strand.) Explain your reasoning.

25. The *mmm* operon, which has sequences *A*, *B*, *C*, and *D* (which may be structural genes or regulatory sequences), encodes enzymes 1 and 2. Mutations in sequences *A*, *B*, *C*, and *D* have the following effects, where a plus sign (+) indicates that the enzyme is synthesized and a minus sign (–) indicates that the enzyme is not synthesized.

Mutation in sequence	mmm absent		mmm present	
	Enzyme 1	**Enzyme 2**	**Enzyme 1**	**Enzyme 2**
No mutation	+	+	–	–

A	–	+	–	–
B	+	+	+	+
C	+	–	–	–
D	–	–	–	–

a. Is the *mmm* operon inducible or repressible?

b. Indicate which sequence (*A*, *B*, *C*, or *D*) is part of the following components of the operon:

Regulator gene	_____
Promoter	_____
Structural gene for enzyme 1	_____
Structural gene for enzyme 2	_____

26. DATA ANALYSIS Ellis Engelsberg and his colleagues examined the regulation of genes taking part in the metabolism of arabinose, a sugar (E. Engelsberg et al. 1965. *Journal of Bacteriology* 90:946–957). Four structural genes encode enzymes that help metabolize arabinose (genes *A*, *B*, *D*, and *E*). An additional sequence *C* is linked to genes *A*, *B*, and *D*. These are in the order *D*-*A*-*B*-*C*. Gene *E* is distant from the other genes. Engelsberg and his colleagues isolated mutations at the *C* sequence that affected the expression of structural genes *A*, *B*, *D*, and *E*. In one set of experiments, they created various genotypes at *A* and *C* and determined whether arabinose isomerase (the enzyme encoded by gene *A*) was produced in the presence or absence of arabinose (the substrate of arabinose isomerase) by cells with these genotypes. Results from this experiment are shown in the following table, where a plus sign (+) indicates that the arabinose isomerase was synthesized and a minus sign (–) indicates that the enzyme was not synthesized.

Genotype	Arabinose absent	Arabinose present
C^+A^+	–	+
C^-A^+	–	–
C^-A^+/C^+A^-	–	+

$C^c A^-/C^- A^+$	+	+

a. On the basis of these results, is the *C* sequence an operator or a regulator gene? Explain your reasoning.
b. Do these experiments suggest that the arabinose operon is negatively or positively controlled? Explain your reasoning.
c. What type of mutation is C^c?

27. In *E. coli*, three structural genes (*A*, *D*, and *E*) encode enzymes A, D, and E, respectively. Sequence *O* is an operator. The sequences are in the order *O-A-D-E* on the chromosome. The three enzymes encoded by *A*, *D*, and *E* catalyze the biosynthesis of valine. Mutations at the *A*, *D*, *E*, and *O* were isolated to study the production of enzymes A, D, and E when cellular levels of valine were low (T. Ramakrishnan and E. A. Adelberg. 1965. *Journal of Bacteriology* 89:654–660). Amounts of the enzymes produced by partial-diploid *E. coli* with various combinations of mutations are shown in the following table.

		Amount of enzyme produced		
	Genotype	**E**	**D**	**A**
1.	$E^+ D^+ A^+ O^+/$ $E^+ D^+ A^+ O^+$	2.40	2.00	3.50
2.	$E^+ D^+ A^+ O^-/$ $E^+ D^+ A^+ O^+$	35.80	38.60	46.80
3.	$E^+ D^- A^+ O^-/$ $E^+ D^+ A^- O^+$	1.80	1.00	47.00
4.	$E^+ D^+ A^- O^-/$ $E^+ D^- A^+ O^+$	35.30	38.00	1.70
5.	$E^- D^+ A^+ O^-/$ $E^+ D^- A^+ O^+$	2.38	38.00	46.70

a. Is the regulator protein that binds to the operator of this operon a repressor (negative control) or an activator (positive control)? Explain your reasoning.
b. Are genes *A*, *D*, and *E* all under the control of operator *O*? Explain your reasoning.
c. Propose an explanation for the small amount of enzyme E produced by genotype 3.

Section 16.3

28. At which level of gene regulation shown in **Figure 16.1** does attenuation occur?

29. *Listed in parts *a* through *g* are some mutations that were found in the 5′ UTR of the *trp* operon of *E. coli*. What will the most likely effect of each of these mutations be on the transcription of the *trp* structural genes?
    a. A mutation that prevents the binding of the ribosome to the 5′ end of the mRNA 5′ UTR
    b. A mutation that changes the Trp codons in region 1 of the mRNA 5′ UTR into codons for alanine
    c. A mutation that creates a stop codon early in region 1 of the mRNA 5′ UTR
    d. Deletions in region 2 of the mRNA 5′ UTR
    e. Deletions in region 3 of the mRNA 5′ UTR
    f. Deletions in region 4 of the mRNA 5′ UTR
    g. Deletion of the string of adenine nucleotides that follows region 4 in the 5′ UTR

30. Some mutations in the *trp* 5′ UTR increase termination by the attenuator. Where might these mutations occur, and how might they affect the attenuator?

31. CONNECT Some of the mutations of the type mentioned in Problem 29 have an interesting property: they prevent the formation of the antiterminator that normally takes place when the tryptophan level is low. In one of these mutations, the AUG start codon for translation of the 5′ UTR has been deleted (see Chapter 15 for a discussion of start codons). How might this mutation prevent antitermination from taking place?

Section 16.4

32. *Several examples of antisense RNA regulating translation in bacterial cells have been discovered. Molecular geneticists have also used antisense RNA to artificially control transcription in both bacterial and eukaryotic genes. If you wanted to inhibit the transcription of a bacterial gene with antisense RNA, what sequences might the antisense RNA contain?

33. Which specific sequences in the 5′ UTR of the *ompF* mRNA do you think *micF* RNA binds? See Chapter 15 for information about ribosome binding and initiation of translation.

CHALLENGE QUESTIONS

Section 16.3

34. Would you expect to see attenuation in the *lac* operon and other operons that control the metabolism of sugars? Why or why not?

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Introduction

1. What are some advantages and disadvantages of using mathematical models to study biological processes such as the function of operons?

Section 16.1

2. What types of genes would you expect to be constitutive?

Section 16.2

3. Suppose that an operon that exhibited positive control was inducible. Would the regulator gene in this case produce an active or an inactive activator protein? What would most likely turn transcription on? What about an operon with positive control that was repressible? In this case, what would turn transcription off? Explain your reasoning.

4. During the development of most mammals, when is lactose available for use by *E. coli* in the gut? How does the availability of lactose to *E. coli* in humans differ from that seen in other mammals?

5. A strain of *E. coli* has the following genotypes at the *lac* operon, where *I* = regulator gene, *P* = promoter, *O* = operator, *Z* = *β*-galactosidase gene, and *Y* = permease gene. The superscript$^+$ indicates a wild-type allele, ^{c} indicates a constitutive mutation, and $^-$ indicates a defective mutation. For each genotype, indicate whether the enzyme will be synthesized or not synthesized when lactose is present or absent by placing a + *for synthesis occurring* and a − *for synthesis not occurring* in the appropriate blank. Explain your reasoning for each answer.

	Inducer (lactose) absent		Inducer (lactose) present	
Genotype	***β*-Galactosidase**	**Permease**	***β*-Galactosidase**	**Permease**
a. $lacI^+\ lacP^+\ lacO^+\ lacZ^+\ lacY^+$	_____	_____	_____	_____
b. $lacI^+\ lacP^+\ lacO^c\ lacZ^+\ lacY^+$	_____	_____	_____	_____
c. $lacI^-\ lacP^+\ lacO^+\ lacZ^-\ lacY^+$	_____	_____	_____	_____
d. $lacI^-\ lacP^+\ lacO^+\ lacZ^+\ lacY^+/lacI^+\ lacP^-\ lacO^+\ lacZ^+\ lacY^-$	_____	_____	_____	_____

e. *lacI*$^-$ *lacP*$^+$ *lacO*^{c} *lacZ*$^-$ *lacY*$^+$/*lacI*$^+$ *lacP*$^+$ *lacO*$^+$ *lacZ*$^+$ *lacY*$^+$	_____	_____	_____	_____
f. *lacI*$^+$ *lacP*$^-$ *lacO*^{c} *lacZ*$^+$ *lacY*$^-$/*lacI*$^-$ *lacP*$^+$ *lacO*$^+$ *lacZ*$^-$ *lacY*$^+$	_____	_____	_____	_____

Section 16.3

6. Indicate whether each of the following events occurs when tryptophan is high or when tryptophan is low by placing a check in each of the appropriate blanks. Share your reasoning.

Event	Tryptophan high	Tryptophan low
Ribosome does not stall at Trp codons	_____	_____
Region 2 of the leader pairs with region 3	_____	_____
Ribosome covers part of region 2 of leader	_____	_____
Transcription is terminated before structural genes are transcribed	_____	_____

7. What would be the effect on tryptophan production of mutations in region 1 of the leader of the *trp* operon that changed the Trp codons to stop codons? Explain your reasoning.

Section 16.4

8. How might antisense RNAs be used as antibiotics?

ACTIVE LEARNING: CONCEPT MAPPING QUESTIONS

Introduction

1. Create a concept map that summarizes research showing that operons may decrease biochemical noise in prokaryotes. Include at least the following names, terms, and phrases: operons, prokaryotic genes, eukaryotic genes, *lac* operon, Jacob, Monod, genes transcribed together, biochemical noise, mathematical modeling, Igoshin, Ray, gene interaction in operons, genes that are actually found in operons.

Section 16.1

2. Draw a concept map that shows the relationships between the following terms: gene regulation, constitutive genes, structural genes, regulatory genes, regulatory elements. Include other terms in your map if needed.

Section 16.2

3. Summarize the parts of the *lac* operon and how the *lac* operon controls the production of *β*-galactosidase and permease.

Section 16.3

4. Summarize in the form of a concept map the process of attenuation in the *trp* operon.

Section 16.4

5. Show the relationships between the following terms that represent structures located outside operons and their function in controlling expression of some bacterial genes: gene expression, bacterial enhancer, antisense RNA, RNA secondary structures, RNA-mediated repression, increased transcription, self-cleavage, translation, ribozymes.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 17

Control of Gene Expression in Eukaryotes

Edwin Hadley, University of Illinois.

Changes in a relatively small number of regulatory sequences help produce the large phenotypic differences between humans and chimpanzees. Illustrated here is a network of interacting genes encoding transcription factors that are differentially expressed in the brains of humans and chimpanzees and control the expression of other genes. Red circles represent transcription factors that are more

highly expressed in the human brain; green circles represent transcription factors that are more highly expressed in the chimpanzee brain.

Description
The background shows a large number of orange circles over the human outline. These circles are interconnected through multiple straight lines. Several tiny green circles are present over the chimpanzee outline and these are also interconnected through straight lines. The orange and green circles between the outlines are also interconnected through straight lines.

Genetic Differences That Make Us Human

Over 140 years ago, Charles Darwin proposed that humans share a recent common ancestor with the African great apes. Today, there is significant evidence that our closest living relative is the chimpanzee (*Pan troglodytes*). Fossil evidence indicates that humans and chimpanzees diverged genetically only 5 million to 7 million years ago—a mere blink of the eye in evolutionary time. Yet humans and chimpanzees differ in a large number of anatomical, physiological, behavioral, and cognitive traits. For example, there are numerous differences in the structure of the backbone, pelvis, skull, jaw, teeth, hands, and feet of humans and chimpanzees. The size of the human brain is more than twice that of the chimpanzee brain, and humans exhibit complex language and cultural characteristics not seen in chimpanzees. Indeed, the degree of phenotypic difference between chimpanzees and humans is so large that scientists in the past

placed them in entirely different primate families (humans in the family Hominidae and chimpanzees in the family Pongidae), although today biologists often put them together in the family Hominidae.

In spite of the large phenotypic gulf between humans and chimpanzees, sequencing of their genomes reveals that their DNA is remarkably similar. There is only a 1% difference in individual base pairs between the two species, along with a 3% difference in insertions and deletions. Thus, 96% of the DNA of humans and chimpanzees is identical. But clearly humans are not chimpanzees. How, then, did humans and chimpanzees come to be so different? Where are the genes that make us human?

A possible answer to this paradox was proposed by geneticists Mary-Claire King and A. C. Wilson in 1975. Using the limited techniques available at the time (comparison of amino acids in proteins and DNA hybridization studies), King and Wilson concluded that humans and chimpanzees differed at only about 1% of their DNA sequences. To explain how these very small genetic changes could account for the extensive physical and behavioral differences between humans and chimpanzees, King and Wilson suggested that the genetic variations that make us human are concentrated in regulatory sequences—those parts of the genome that control the expression of other genes. In this way, small genetic changes might influence the expression of numerous other genes and affect the phenotypes of many traits simultaneously. Unfortunately, there were no techniques

available at the time to examine regulatory sequences to test their hypothesis.

Fast-forward to 2009. Using cutting-edge techniques of genomic research and bioinformatics, Katja Nowick and her colleagues at the University of Illinois and the Norwegian University of Science and Technology identified a group of 90 transcription factors whose expression differed significantly between humans and chimpanzees. As discussed in Chapter 13, transcription factors are proteins that bind to DNA and facilitate or repress the synthesis of RNA, the first step in the process of information transfer from genotype to phenotype. Each transcription factor may affect the expression of multiple genes, so a small genetic change affecting the expression of a single transcription factor can influence many additional genes. The differences that Nowick and her colleagues found in the expression of transcription factors were particularly pronounced in brain tissue, where they may account for the large differences in neural and cognitive function between humans and chimpanzees.

Many of the transcription factors that Nowick and her colleagues identified were Krüppel-associated box domain zinc finger proteins (KRAB-ZFPs), transcription factors that bind to specific DNA sequences and bring about changes in chromatin structure. As we'll see in this chapter, changes in chromatin structure are often involved in regulation of gene expression in eukaryotes. Other studies have demonstrated that KRAB-ZFPs have evolved rapidly in humans, probably because they were favored by natural selection.

The transcription factors identified by Nowick and colleagues clustered into two distinct but interconnected regulatory networks, which control energy metabolism, transcription, vesicular transport, and protein modification.

These results support the idea first proposed by King and Wilson: that changes in a relatively small number of regulatory sequences affect the expression of numerous genes in humans and chimpanzees and help produce the large differences we see in their anatomy, brain size, cognition, and behavior.

THINK-PAIR-SHARE

- Based on their anatomical and behavioral differences, biologists previously placed humans and chimpanzees in different families, but now DNA sequence data suggest that they are closely related and should be placed in the same family. What are some advantages of using DNA sequences to decide how to classify organisms? What might be some disadvantages?
- If 4% of human and chimpanzee DNA differs, how many base pairs differ between the two species? (Hint: The human genome contains 3.2 billion base pairs of DNA.) Is this a large or a small difference? Explain your reasoning.

This chapter is about gene regulation in eukaryotic cells—the very changes that help make humans unique. Gene regulation in eukaryotes typically takes place at multiple levels. We begin by considering how gene expression is influenced by changes in chromatin structure, which can be altered by several different mechanisms. We next consider the initiation of transcription in eukaryotes, which is controlled by the complex interactions of

several types of proteins and the DNA regulatory elements to which they bind. We examine several ways in which gene expression is controlled through the processing, degradation, and translation of mRNA. We end by revisiting some of the similarities and differences in gene regulation in bacteria and eukaryotes.

17.1 Eukaryotic Cells and Bacteria Share Many Features of Gene Regulation but Differ in Several Important Ways

As discussed in Chapter 16, many features of gene regulation are shared by bacterial and eukaryotic cells. For example, in both types of cells, DNA-binding proteins influence the ability of RNA polymerase to initiate transcription. However, there are also some differences. First, many bacterial and archaeal genes are organized into operons and are transcribed into a single RNA molecule. Although some operon-like gene clusters have been found in worms and even in some primitive chordates, most eukaryotic genes have their own promoters and are transcribed separately. Second, chromatin structure affects gene expression in eukaryotic cells; DNA must unwind from the histone proteins before transcription can take place. Third, the presence of the nuclear membrane in eukaryotic cells separates transcription and translation in time and space. Therefore, the regulation of gene expression in eukaryotic cells is characterized by a greater diversity of mechanisms that act at different points in the transfer of information from DNA to protein.

17.2 Changes in Chromatin Structure Affect the Expression of Eukaryotic Genes

One type of gene control in eukaryotic cells is accomplished through the modification of chromatin structure. In the nucleus, histone proteins associate to form octamers, around which helical DNA tightly coils to create chromatin (see Figure 11.4). In a general sense, this chromatin structure itself represses gene expression. For a gene to be transcribed, proteins called transcription factors (see Section 17.3) must bind to the DNA. Other regulator proteins and RNA polymerase must also bind to the DNA for transcription to take place. How can these proteins bind with DNA wrapped tightly around histone proteins? The answer is that before transcription, chromatin structure changes so that the DNA becomes more accessible to the transcription machinery.

DNase I Hypersensitivity

As genes become transcriptionally active, regions around the genes become highly sensitive to the action of DNase I (see pp. 323–324 in Chapter 11). These regions, called **DNase I hypersensitive sites**, frequently develop about 1000 nucleotides upstream of the transcription start site, suggesting that the chromatin in these

regions adopts a more open configuration during transcription. This relaxation of the chromatin structure allows regulatory proteins access to binding sites on the DNA. Indeed, many DNase I hypersensitive sites correspond to known binding sites for regulatory proteins. At least three different processes affect gene regulation by altering chromatin structure: (1) chromatin remodeling, (2) the modification of histone proteins, and (3) DNA methylation. Each of these mechanisms will be discussed in the sections that follow.

THINK-PAIR-SHARE Question 1

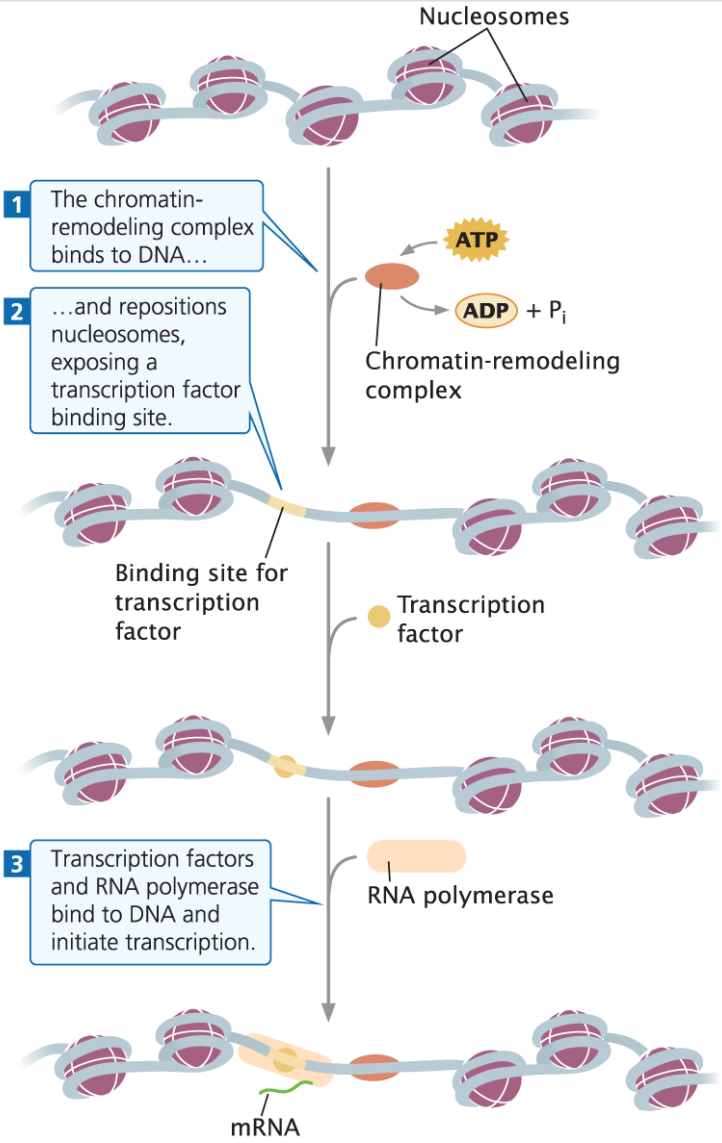
CONCEPTS

Sensitivity to DNase I in regions around transcriptionally active genes indicates that the chromatin in these regions assumes an open configuration before transcription.

Chromatin Remodeling

Some transcription factors and other regulatory proteins alter chromatin structure without altering the chemical structure of the histones directly. These proteins are called **chromatin-remodeling complexes**. They bind directly to particular sites on DNA and reposition the nucleosomes, allowing other transcription factors and

RNA polymerase to bind to promoters and initiate transcription (Figure 17.1).



17.1 Chromatin-remodeling complexes reposition the nucleosomes, allowing transcription factors and RNA polymerase to bind to promoters and initiate transcription.

Description

The illustration shows a string of nucleosomes with D N A wrapped around it at the top. Below an A T P molecule interacts with a Chromatin-remodeling complex and an A D P molecule and P subscript small I are released. This chromatin-remodeling complex binds to the D N A below. There is a considerable space in the center of the string with 2 nucleosomes on its left side and 3 on its right side. A binding site for transcription factor is on the left of the Chromatin-remodeling complex. A caption reads, the chromatin remodeling complex binds to D N A and repositions nucleosomes, exposing a transcription factor binding site. A transcription factor enters the process and binds at the binding site below. An R N A polymerase enters the process below and attaches to the transcription factor. An m R N A is formed below, that extends out of the polymerase molecule. A caption reads, Transcription factors and R N A polymerase bind to D N A and initiate transcription.

One of the best-studied examples of a chromatin-remodeling complex is SWI–SNF, which is found in yeast, humans, *Drosophila*, and other eukaryotes. This complex uses energy derived from the hydrolysis of ATP to reposition nucleosomes, exposing promoters in the DNA to the action of other regulatory proteins and RNA polymerase.

Evidence suggests at least two mechanisms by which chromatin-remodeling complexes reposition nucleosomes. First, some complexes cause the nucleosome to slide along the DNA, allowing DNA that was wrapped around the nucleosome to occupy a position in between nucleosomes, where it is more accessible to proteins affecting gene expression (see Figure 17.1). Second, some complexes cause conformational changes in the DNA, in nucleosomes, or in both so that DNA that is bound to the nucleosome assumes a more exposed configuration.

Chromatin-remodeling complexes are targeted to specific DNA sequences by transcription factors that attach to a complex and then bind to the promoters of specific genes. There is also evidence that chromatin-remodeling complexes work together with enzymes that alter histones, such as acetyltransferase enzymes (which we'll describe shortly), to change chromatin structure and expose DNA for transcription.

THINK-PAIR-SHARE Question 2

Histone Modification

The histones in the octamer core of a nucleosome have two domains: (1) a globular domain that associates with the other histones and the DNA, and (2) a positively charged tail domain that interacts with the negatively charged phosphate groups on the DNA. The tails of histone proteins are often modified by the addition or removal of phosphate groups, methyl groups, or acetyl groups. Another modification of histones is ubiquitination, in which a small molecule called ubiquitin is added to or removed from the histones. These modifications have sometimes been collectively called the **histone code** because they encode information that affects how genes are expressed. The histone code affects gene expression by altering chromatin structure directly or, in some cases, by providing

recognition sites for proteins that bind to DNA and regulate transcription.

METHYLATION OF HISTONES

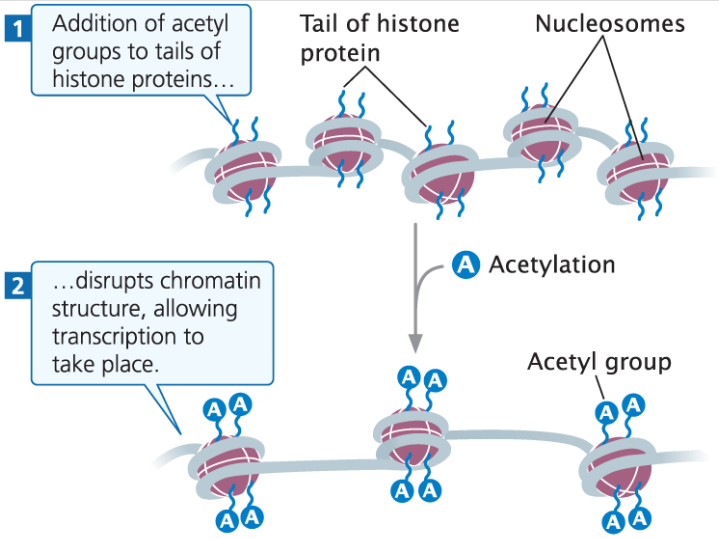
One type of histone modification is the addition of methyl groups (CH_3) to the tails of histone proteins. These modifications can bring about either the activation or the repression of transcription, depending on which histone is modified and which particular amino acids in the histone tail are methylated. Enzymes called histone methyltransferases add methyl groups to specific amino acids (usually lysine or arginine) of histones. Other enzymes, called histone demethylases, remove methyl groups from histones. Many of the enzymes and proteins that modify histones, such as histone methyltransferases and demethylases, do not bind to specific DNA sequences and must be recruited to specific chromatin sites. Sequence-specific binding proteins, preexisting histone modifications, and RNA molecules serve to recruit histone-modifying enzymes to specific sites.

A common modification is the addition of three methyl groups to lysine 4 in the tail of the H3 histone protein, abbreviated H3K4me3 (K is the abbreviation for lysine). Histones containing the H3K4me3 modification are frequently found near promoters of transcriptionally active genes. Studies have identified proteins that recognize and bind to H3K4me3, including nucleosome remodeling factor (NURF). NURF and other proteins that recognize H3K4me3

have a common protein-binding domain that binds to the H3 histone tail and then alters chromatin packing, allowing transcription to take place. Research has also demonstrated that some transcription factors that are necessary for the initiation of transcription (see Chapter 13 and Section 17.3) bind directly to H3K4me3.

ACETYLATION OF HISTONES

Another type of histone modification that affects chromatin structure is acetylation, the addition of acetyl groups (CH_3CO) to histones. The acetylation of histones usually stimulates transcription. For example, the addition of a single acetyl group to lysine 16 in the tail of the H4 histone prevents the formation of the 30-nm chromatin fiber (see Figure 11.4), causing the chromatin to be in an open configuration that makes the DNA available for transcription. In general, acetyl groups destabilize chromatin structure, allowing transcription to take place (**Figure 17.2**). Acetyl groups are added to histone proteins by acetyltransferase enzymes; other enzymes called deacetylases strip acetyl groups from histones and restore chromatin structure, which represses transcription. Certain transcription factors (see Chapter 13) and other proteins that regulate transcription either have acetyltransferase activity themselves or attract acetyltransferases to DNA.



17.2 The acetylation of histone proteins alters chromatin structure.

Description

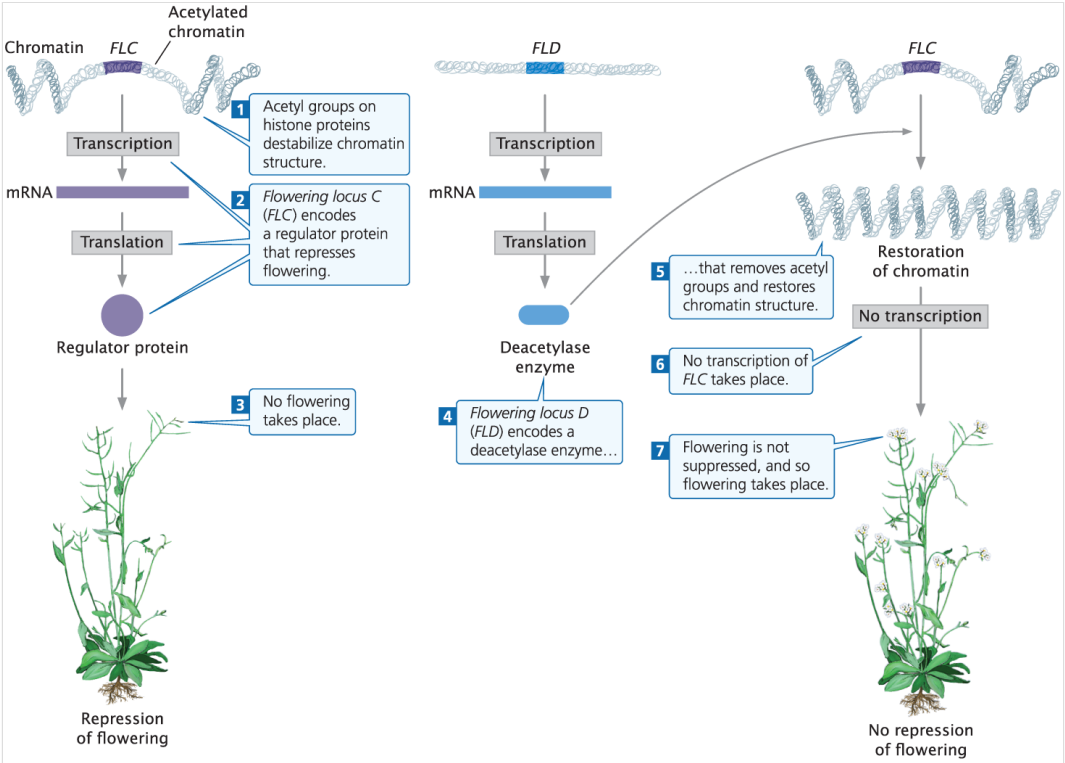
The illustration shows a string of nucleosomes with D N A wrapped around it. Each nucleosome has two wavy lines extending at the top and two wavy lines extending at the bottom. These lines are labeled as tail of histone protein. A caption reads, Addition of acetyl groups to tails of histone proteins. Acetylation occurs below. An acetyl group, represented by big A, binds to each of the histone tail, for all the nucleosomes. A caption reads, disrupts chromatin structure, allowing transcription to take place.

ACETYLATION OF HISTONES AND FLOWERING IN *ARABIDOPSIS*

The importance of histone acetylation in gene regulation is demonstrated by the control of flowering in *Arabidopsis thaliana*, a plant with a number of characteristics that make it an excellent genetic model for plant systems (see the Reference Guide to Model

Genetic Organisms at the end of this book). The time at which flowering takes place is critical to the life of a plant: if flowering is initiated at the wrong time of year, pollinators may not be available to fertilize the flowers, or environmental conditions may be unsuitable for the survival and germination of the seeds. Consequently, flowering time in most plants is carefully regulated in response to multiple internal and external cues, such as plant size, photoperiod, and temperature.

Among the many genes that control flowering in *Arabidopsis* is *flowering locus C* (*FLC*), which plays an important role in suppressing flowering until after an extended period of cold (a process called vernalization). The *FLC* gene encodes a regulatory protein (a transcription factor) that represses the activity of other genes that affect flowering (**Figure 17.3**). As long as *FLC* is transcriptionally active, flowering remains suppressed.



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17.3 Flowering in *Arabidopsis* is controlled in part by *FLD*, a gene that encodes a deacetylase enzyme. This enzyme removes acetyl groups from histone proteins in the chromatin surrounding *FLC*, a gene that suppresses flowering. The removal of the acetyl groups from the histones restores chromatin structure and represses the transcription of *FLC*, thereby allowing the plant to flower.

Description

The illustration shows a coiled, chromatin, on the left side, with a straight part in its center. This part has a gene, big F big L big C. Transcription occurs and a single stranded m R N A is obtained below. A caption reads, acetyl groups on histone proteins destabilize chromatin structure. Translation occurs and a circular shaped protein is formed below. A caption reads, flowering locus C (big F big L big C) encodes a regulator protein that represses flowering. A plant below has no flowers. A caption reads, no flowering takes place. Text below reads, repression of flowering.

The illustration shows a straight chromatin, at the center, with a gene big F big L big D at the chromatin's center. Transcription occurs and a single stranded m R N A is obtained

below. Translation occurs and a Deacetylase enzyme is formed below. A caption reads, Flowering locus D (big F big L big D) encodes a deacetylase enzyme.

The illustration shows a coiled chromatin on the right, with a straight part in its center. This part has a gene, big F big L big C. The chromatin restores its coiled shape below with no straight part in the center, in the presence of the deacetylase enzyme. A caption reads, that removes acetyl groups and restores chromatin structure. No transcription takes place. A caption reads, no transcription of big F big L big C takes place. A plant below has white flowers. A caption reads, flowering is not suppressed, and so flowering takes place. Text below reads, No repression of flowering.

The activity of *FLC* is controlled by another locus called *flowering locus D* (*FLD*), the key role of which is to stimulate flowering by repressing the action of *FLC*. In essence, flowering is stimulated because *FLD* represses the repressor. How does *FLD* repress *FLC*? *FLD* encodes a deacetylase enzyme, which removes acetyl groups from histone proteins in the chromatin surrounding *FLC* (see Figure 17.3). The removal of these acetyl groups alters the chromatin structure and inhibits transcription. The inhibition of transcription prevents *FLC* from being transcribed and removes its repression on flowering. In short, *FLD* stimulates flowering in *Arabidopsis* by deacetylating the chromatin that surrounds *FLC*, thereby removing its inhibitory effect on flowering. › **TRY PROBLEM 19**

CHROMATIN IMMUNOPRECIPITATION

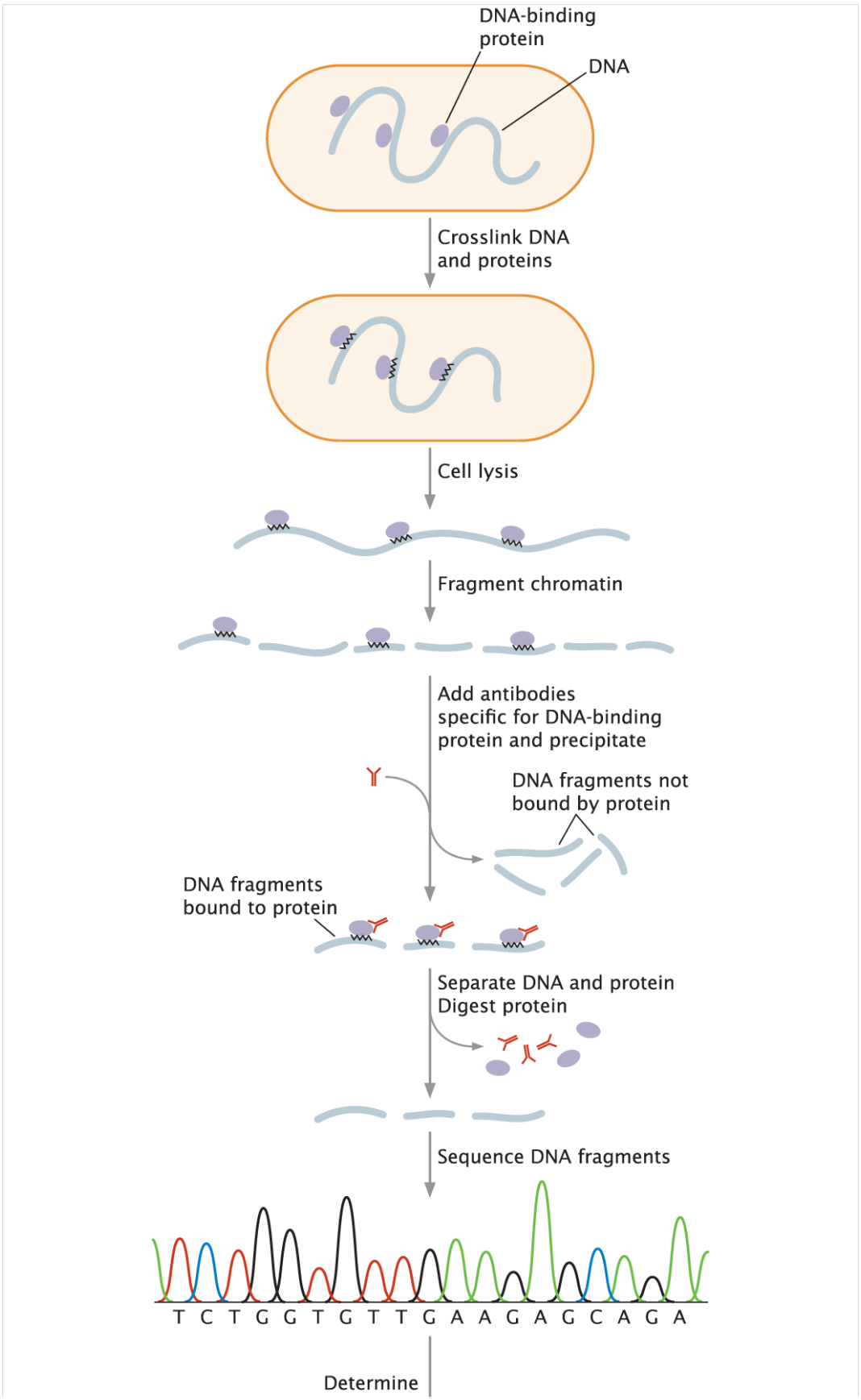
Our understanding of how changes in chromatin structure are associated with gene expression, and of how DNA-binding proteins

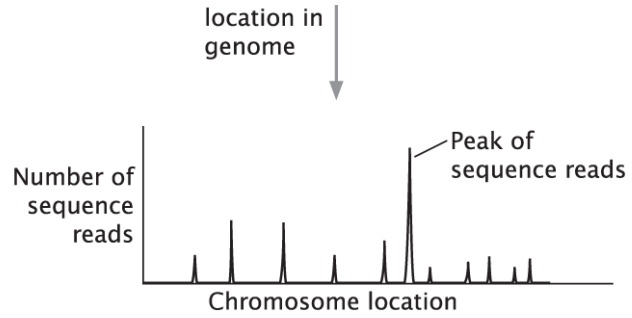
affect transcription, has been greatly advanced by the use of a technique called chromatin immunoprecipitation (ChIP). This technique allows researchers to determine the locations within the genome where a specific protein interacts with DNA. ChIP has been used to determine the locations of histones that have undergone modifications as well as where transcription factors and other proteins bind to promoters and enhancers.

The basic idea of ChIP is that a particular protein and the DNA to which it is bound are isolated, the protein and DNA are then separated, and the DNA sequence to which the protein was formerly bound is identified. The technique has provided a powerful means of determining the genome-wide locations of modified histones and the binding sites for transcription factors and other proteins that affect transcription.

One version of ChIP, called crosslinked ChIP (XChIP), is used for identifying the binding sites of transcription factors and other proteins that bind to DNA. In this procedure (**Figure 17.4**), the protein and associated DNA are temporarily crosslinked, which means that they are treated with formaldehyde or UV light to create covalent bonds between the DNA and protein. The crosslinking holds the DNA and protein together so that the DNA to which the protein is bound can be isolated along with the protein. After crosslinking, the cell is lysed, and the chromatin is broken into pieces by digestion with an enzyme or by mechanical shearing. Antibodies specific for a particular protein—such as a specific

transcription factor—are then applied. The antibodies attach to the protein–DNA complexes as well as to a solid substrate (usually small beads). These beads can then be used to precipitate the protein–DNA complexes. Next, the crosslinking is reversed, separating the DNA and the protein. The protein is removed by an enzyme that digests protein but not DNA, leaving fragments of the DNA to which the protein was bound.


DNA-binding protein
DNA
Crosslink DNA and proteins
Cell lysis
Fragment chromatin
Add antibodies specific for DNA-binding protein and precipitate
DNA fragments not bound by protein
DNA fragments bound to protein
Separate DNA and protein
Digest protein
Sequence DNA fragments
T C T G G T G T T G A A G A G C A G A
Determine



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17.4 Chromatin immunoprecipitation (ChIP) can be used to identify the DNA binding sites of a specific protein and the locations of modified histone proteins. Shown here is the method for crosslinked chromatin immunoprecipitation (XChIP).

Description

The illustration shows a rod-shaped cell with a wavy D N A strand in the center. Three tiny oval structures are bound to D N A and are labeled D N A binding protein. Crosslink D N A and proteins enter the process. The tiny zig-zag shaped crosslink D N A binds to the D N A binding protein below. Cell lysis occurs and a slightly wavy D N A strand is obtained, with D N A binding proteins and crosslink D N A strands. Fragment the chromatin. The D N A strand is now divided into various fragments below. Add antibodies specific for D N A-binding protein and precipitate. The D N A fragments that are not bound by protein are eliminated and D N A fragments that are bound to protein are selected below. An antibody gets attached to each of these protein bound fragment. Separate D N A and protein. Digest protein. The antibodies and the D N A binding protein are eliminated. D N A fragments are obtained below. These D N A fragments are sequenced and a graph with overlapping peaks of varying heights is obtained below. The sequence obtained from left to right is as follows: T, C, T, G, G, T, G, T, T, G, A, A, G, A, G, C, A, G, A. Determine location in genome. A graph obtained below shows chromosome location on the horizontal axis and number of sequence reads on the vertical axis. The highest peak obtained is labeled peak of sequence reads.

The location of the resulting DNA fragments within the genome can then be determined by several different methods. One method,

termed ChIP-Seq, determines the base sequences of the fragments using next-generation sequencing technologies (see Chapter 19). When the chromatin is broken into pieces and subjected to immunoprecipitation (see Figure 17.4), many short overlapping fragments of DNA are precipitated. Some of the sequences within each fragment are those that were covered by the protein; others are DNA on either side of the binding site. When the overlapping fragments are sequenced, the base pairs that were covered by the protein will be present in more copies and will be sequenced more often (resulting in more sequence reads) than base pairs that were not covered by the protein, producing peaks of sequence reads at those sites where the protein was bound. The locations of these peaks within the genome can be determined by comparing the sequences within the peaks with a reference genome sequence. The results provide information about the genomic locations of binding sites for the specific protein.

Another version of ChIP, called native ChIP (nChIP), does not use crosslinking. It is often used for finding the locations of modified histone proteins. In this case, crosslinking is not required because the DNA and the histones are naturally linked by the nucleosome structure. The chromatin is isolated from the cell and fragmented, and antibodies to a particular protein—usually a specific modified histone—are used to precipitate the protein–DNA complexes. The histones and DNA are separated, the histones are digested, and the DNA fragments to which the modified histones were attached are sequenced or otherwise identified.

ChIP analysis has been used to determine the locations of modified histones that activate or repress transcription. As mentioned earlier, the H3K4me3 histone modification is associated with promoters of active genes. ChIP analysis has successfully identified locations of this modified histone in the human genome, helping identify active promoters in different tissues.

CONCEPTS

The tails of histone proteins are often modified by the addition or removal of phosphate groups, methyl groups, or acetyl groups. These modifications alter chromatin structure and affect the transcription of genes. Chromatin immunoprecipitation can be used to identify the locations within the genome of modified histones and binding sites for regulatory proteins.

DNA Methylation

Another change in chromatin structure associated with transcription is the methylation of cytosine bases, which yields 5-methylcytosine (see Figure 10.20). The methylation of cytosine in DNA is distinct from the methylation of histone proteins mentioned earlier. Heavily methylated DNA is associated with the repression of transcription in vertebrates and plants, whereas transcriptionally active DNA is usually unmethylated in these organisms. Abnormal patterns of methylation are also associated with some types of cancer.

DNA methylation is most common on cytosine bases adjacent to guanine nucleotides (CpG, where p represents the phosphate group in the DNA backbone), so two methylated cytosines sit diagonally across from each other on opposite strands:

$$\begin{array}{l} 5'-\overset{\text{m}}{\text{C}}\,\text{G}-3' \\ 3'-\text{G}\,\underset{\text{m}}{\text{C}}-5' \end{array}$$

DNA regions with many CpG sequences are called **CpG islands** and are commonly found near transcription start sites. While genes are not being transcribed, these CpG islands are often methylated, but the methyl groups are removed before the initiation of transcription. CpG methylation is also associated with long-term gene repression, such as that of the inactivated X chromosome of female mammals (see Chapter 4).

An association exists between DNA methylation and the deacetylation of histones, both of which repress transcription. Certain proteins that bind tightly to methylated CpG sequences form complexes with other proteins that act as histone deacetylases. In other words, methylation attracts deacetylases, which remove acetyl groups from the histone tails, stabilizing the nucleosome structure and repressing transcription. Demethylation of DNA allows acetyltransferases to add acetyl groups, disrupting nucleosome structure and permitting transcription.

Changes in chromatin structure that affect gene expression, such as the mechanisms just described (chromatin remodeling, histone modification, and DNA methylation), are examples of the phenomenon of epigenetics: alterations to DNA and chromatin structure that affect traits and are passed on to other cells or generations but are not caused by changes in the DNA base sequence. Epigenetic changes and their effects on gene regulation will be discussed in more detail in Chapter 21. ❯ **TRY PROBLEM 20**

CONCEPTS

Chromatin structure can be altered by methylation of DNA. In eukaryotes, DNA methylation often results in 5-methylcytosine at CpG sequences. DNA methylation is usually associated with repression of transcription.

✔ CONCEPT CHECK 1

What are some of the processes that affect gene regulation by altering chromatin structure?

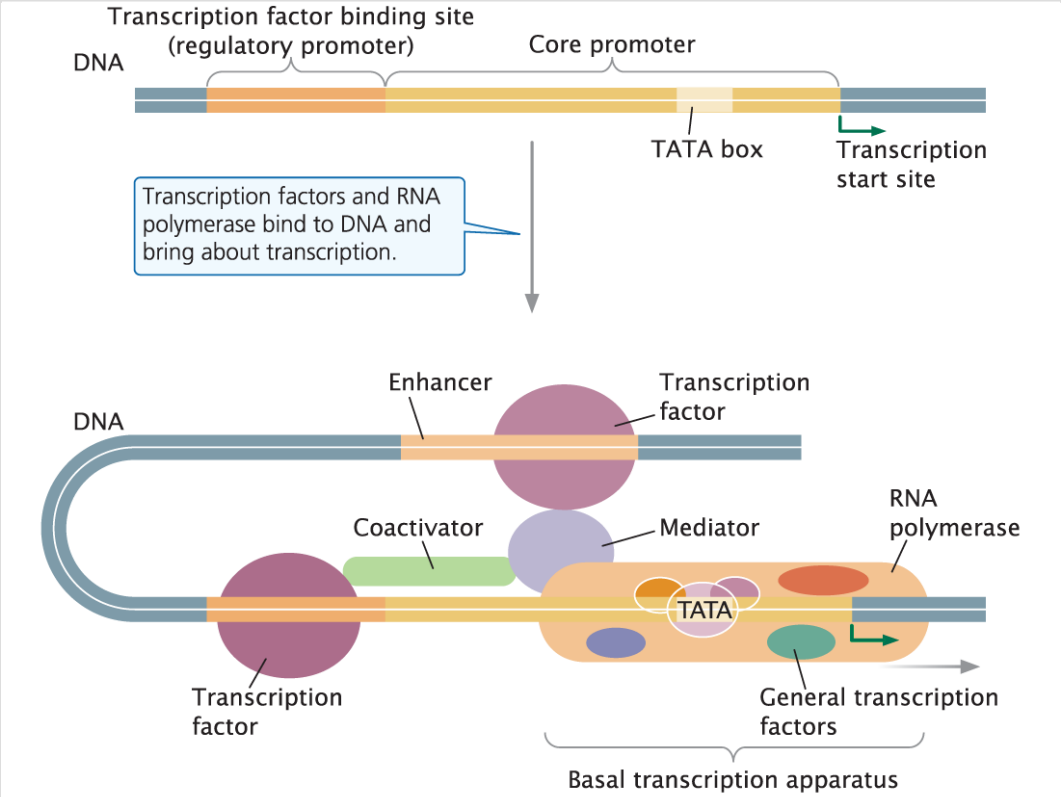
17.3 Transcription Is Regulated by Transcription Factors

We have just considered one level at which gene expression is controlled: the alteration of chromatin and DNA structure. We now turn to another important level of control: control through the binding of proteins to DNA sequences that affect transcription. Transcription is an important level of control in eukaryotic cells, and its control requires a number of different types of proteins and regulatory elements.

Transcription Factors

Eukaryotic transcription was discussed in detail in Chapter 13. Recall that transcription in eukaryotes is regulated by proteins called *transcription factors* that bind to specific DNA sequences; for this function, most transcription factors contain one or more DNA-binding motifs, such as the helix-turn-helix, zinc finger, or leucine zipper (see Figure 16.2). Many transcription factors regulate transcription by recruiting other proteins, called cofactors, which either stimulate or repress transcription. *General transcription factors* bind to the core promoter and are part of the *basal transcription apparatus*—the complex of RNA polymerase, transcription factors, and other proteins that assemble to carry out transcription. General

transcription factors are required for initiation of transcription. The basal transcription apparatus binds to a *core promoter* located immediately upstream of a gene and is capable of minimal levels of transcription. Other transcription factors bind to a *regulatory promoter*, which is located upstream of the core promoter (Figure 17.5), and to *enhancers*, which may be located some distance from the gene. Some transcription factors are activators, stimulating transcription; others are repressors, inhibiting transcription.



17.5 Transcription factors bind to sites on DNA and regulate transcription.

Description

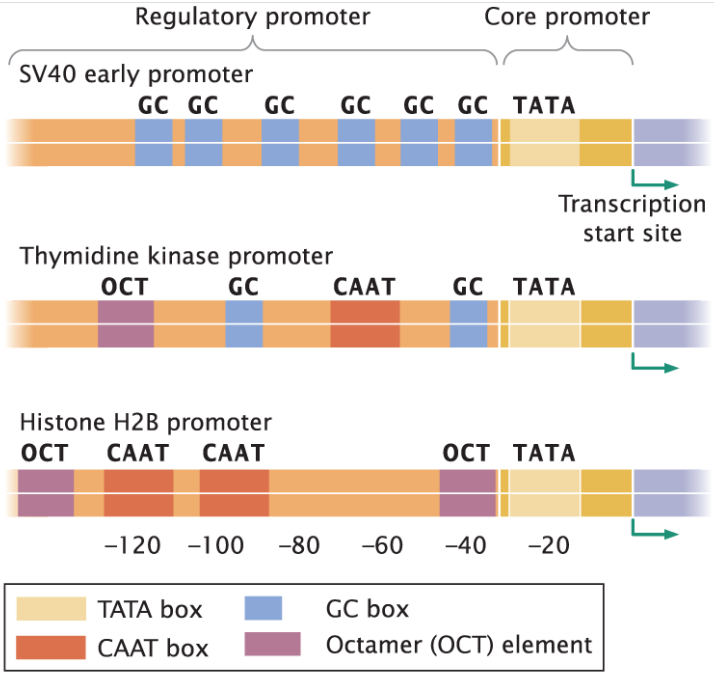
The illustration shows two D N A fragments. The first fragment at the top is a linear D N A fragment with regions labeled. The following regions are labeled from left to right:

Transcription factor binding site or regulatory promoter, the core promoter with a T A T A box, and the transcription start site. An arrow points to the picture of the D N A fragment with transcription factors attached. A caption reads, Transcription factors and R N A polymerase bind to D N A and bring about transcription.

A transcription activator factor is bound on a region labeled enhancer, which is upstream from the promoter. Another transcription factor is bound to the transcription factor binding site. An R N A polymerase and various smaller transcription factors surround the T A T A box and transcription start site. Between the polymerase and transcription factor, a co-activator and a mediator protein are present. Attachment of the Transcription factor to the enhancer, which is further bound to the mediator, causes the D N A to form a loop.

Some transcription factors stimulate and stabilize the basal transcription apparatus at the core promoter. These transcription factors may interact directly with the basal transcription apparatus or act on it indirectly through coactivator proteins. Others facilitate or repress transcription by recruiting enzymes and proteins that alter chromatin structure (see Section 17.2).

Regulatory promoters typically contain several different consensus sequences to which different transcription factors can bind. In different promoters, transcription factor binding sites are mixed and matched in different combinations (**Figure 17.6**), so each promoter is regulated by a unique combination of transcription factors.



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17.6 The consensus sequences in the promoters of three eukaryotic genes illustrate the principle that these sequences are mixed and matched in different combinations in different promoters. Different transcription factors bind to each consensus sequence, so each promoter responds to a unique combination of transcription factors.

Description

The illustration shows S V 40 early promoter at the top. The regulatory promoter part has the following from left to right: Six G, C boxes. The core promoter part has the following from left to right: T A T A box and transcription start site.

Thymidine kinase promoter is shown below. The regulatory promoter part has the following from left to right: octamer (O C T) element, G C box, C A A T box, G C box. The core promoter part has the following from left to right: T A T A box and transcription start site.

Histone H 2 B promoter is shown below. The regulatory promoter part has the following from left to right: octamer (O C T) element, C A A T box (negative 120), C A A T box (negative 100), octamer (O C T) element (negative 40). The core promoter part has the following from left to right: T A T A box and transcription start site.

The binding of transcription factors to the consensus sequences in the regulatory promoter affects the assembly or stability of the basal transcription apparatus at the core promoter. One of the components of the basal transcription apparatus is a complex of proteins called the **mediator** (see Figure 17.5), which interacts with RNA polymerase. Transcription factors that bind to sequences in the regulatory promoter (or enhancer) make contact with the mediator and affect the rate at which transcription is initiated.

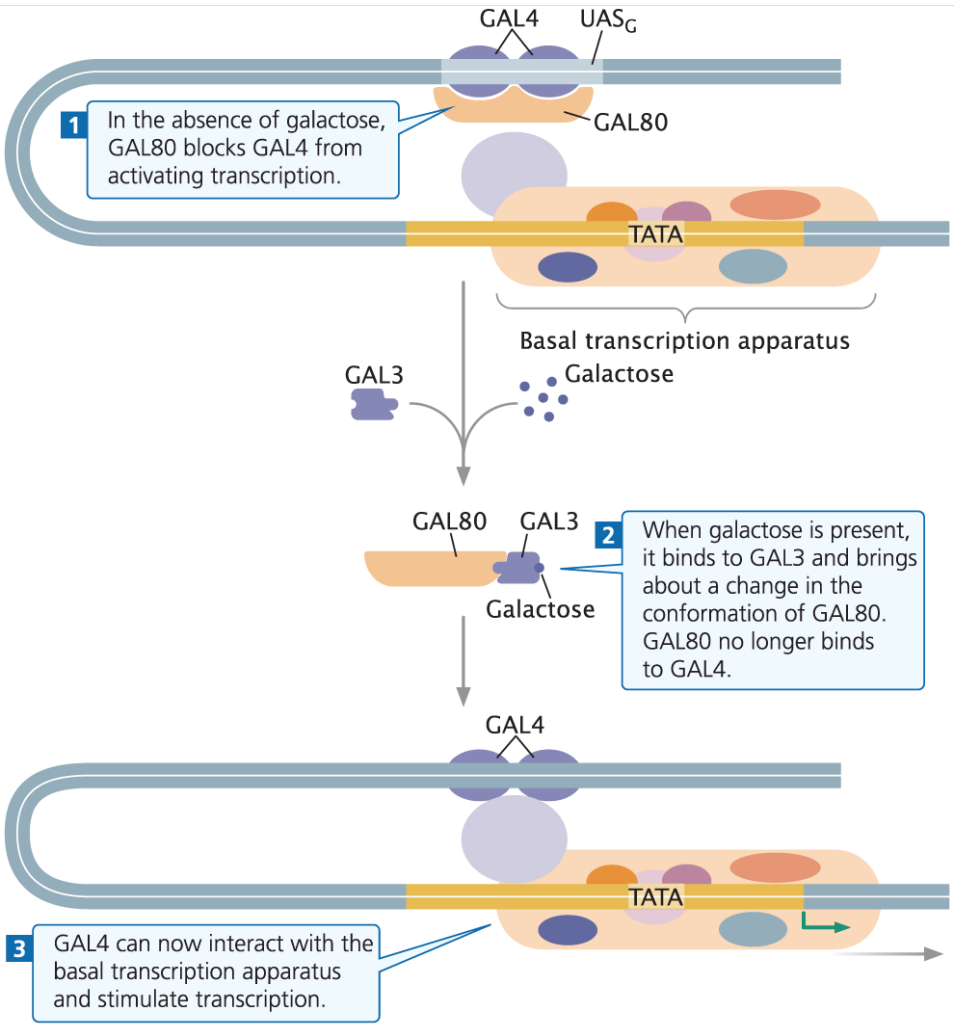
Some regulatory promoters contain sequences that are bound by transcription factors that lower the rate of transcription. Unlike repressors in bacteria, most eukaryotic repressors do not directly block RNA polymerase. These repressors may compete with other transcription factors that activate transcription for binding sites on the DNA: when a site is occupied by the activator, transcription is activated, but if a repressor occupies that site, there is no activation. Alternatively, a repressor may bind to sites near an activating transcription factor and prevent it from contacting the basal transcription apparatus. A third mechanism of repressor action is direct interference with the assembly of the basal transcription apparatus, thereby blocking the initiation of transcription.

THINK-PAIR-SHARE Questions 3 and 4

REGULATION OF GALACTOSE METABOLISM THROUGH GAL4

An example of a transcription factor influencing gene expression is GAL4, which regulates the transcription of several yeast genes whose products metabolize galactose. Like the genes in the *lac* operon, the genes that control galactose metabolism are inducible: when galactose is absent, these genes are not transcribed and the proteins that break down galactose are not produced; when galactose is present, the genes are transcribed and the enzymes are synthesized. GAL4 contains several zinc fingers (DNA-binding motifs) and binds to a DNA sequence called UAS_G (upstream activating sequence for GAL4). UAS_G exhibits the properties of an enhancer—a regulatory sequence that lies some distance from the regulated gene. When bound to UAS_G, GAL4 activates the transcription of yeast genes needed for metabolizing galactose. GAL4 and a number of other transcription factors contain multiple amino acids with negative charges that form an *acidic activation domain*. These acidic activators stimulate transcription by enhancing the assembly of the basal transcription apparatus.

A particular region of GAL4 binds another protein called GAL80, which regulates the activity of GAL4 in the presence of galactose. When galactose is absent, GAL80 binds to GAL4, preventing GAL4 from activating transcription (**Figure 17.7**). When galactose is present, however, the sugar binds to another protein called GAL3, which interacts with GAL80, causing a conformational change in GAL80 so that it can no longer bind GAL4. The GAL4 protein is then free to bind to UAS_G and activate the transcription of the genes whose products metabolize galactose.



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17.7 Transcription is activated by GAL4 in response to galactose. GAL4 binds to the UAS_G site and controls the transcription of genes in galactose metabolism.

Description

The illustration shows a D N A double strand with a loop on the left side. A basal transcription apparatus with several transcription factors is bound to the T A T A box. One G A L 80 binds to two G A L 4. These two G A L 4 are bound to the big U big A big S subscript big G gene, which is upstream the T A TA box. A caption reads, In the absence of galactose, G A L 80 blocks G A L 4 from activating transcription. G A L 3 and several tiny structures labeled galactose enter the process. G A L 80 binds to the G A L 3 which is further bound to one galactose. A caption reads, When galactose is present, it binds to G A L 3 and brings about a change in the conformation of G A L 80. G A L 80 no longer binds to

G A L 4. G A L 80, dissociates from G A L 4, after binding with G A L 3 and galactose. G A L 4 below interacts with the basal transcription apparatus. A caption reads, G A L 4 can now interact with the basal transcription apparatus and stimulate transcription.

CONCEPTS

Transcription factors in eukaryotic cells can influence the initiation of transcription by affecting the stability or assembly of the basal transcription apparatus or by recruiting coactivators that alter chromatin structure. Some transcription factors are activators that stimulate transcription; others are repressors that inhibit the initiation of transcription.

✔ CONCEPT CHECK 2

Many transcription factors stimulate transcription by interacting with

a. introns.
b. the basal transcription apparatus.
c. DNA polymerase.
d. the terminator.

Enhancers and Silencers

Enhancers are regulatory elements that affect the transcription of distant genes. For example, an enhancer that regulates the gene encoding the alpha chain of the T-cell receptor is located 69,000 bp downstream of the gene's promoter; some vertebrate enhancers act over distances of millions of base pairs. The exact position and

orientation of an enhancer relative to the promoter it regulates are not critical to its function (see Chapter 13).

In many eukaryotes, enhancers are highly abundant. For example, it is estimated that there are hundreds of thousands of enhancers in the human genome, compared to approximately 20,000 genes. Enhancers are often a few hundred base pairs in length and contain multiple binding sites for transcription factors; a typical enhancer contains about 10 binding sites for proteins that regulate transcription. Enhancers often regulate genes in a cell type–specific manner, meaning that they turn on different sets of genes in different cell types and help establish the traits that characterize cells of different tissues. Some enhancer-like elements are found in prokaryotes.

How can an enhancer affect the initiation of transcription at a promoter that is tens of thousands of base pairs away? In many cases, the binding of transcription factors to the enhancer causes the DNA between the enhancer and the promoter to loop out, bringing the promoter and enhancer close to each other, so that the transcription factors are able to interact directly with the basal transcription apparatus at the core promoter (see Figure 17.5). Some enhancers may be attracted to promoters by proteins that bind to sequences in the regulatory promoter and "tether" the enhancer close to the core promoter. Enhancers may also affect transcription by undergoing modifications that alter chromatin structure.

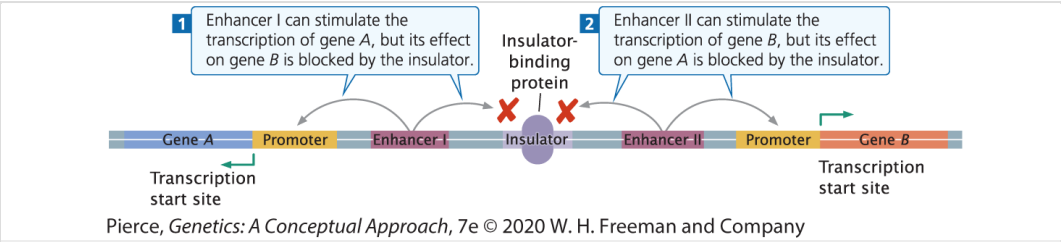
Many enhancers are themselves transcribed into short RNA molecules called enhancer RNAs (eRNAs). Enhancer RNA is often produced bidirectionally, which means that RNA polymerase transcribes eRNA in both directions, copying a different DNA strand in each direction. These RNA molecules do not encode proteins, but transcription of eRNAs may play a role in enhancer function. Transcription of enhancers is often associated with transcription at the promoters that the enhancers affect. How transcription at an enhancer might affect transcription occurring at a distant promoter is not certain. The enhancer might recruit RNA polymerase, which might then be transferred to the promoter when the enhancer interacts with the promoter. Alternatively, transcription of the enhancer might allow the chromatin to adopt a more open configuration, which would then facilitate transcription at nearby promoters. The existence of eRNAs might also represent nothing more than transcriptional noise: because the enhancer is physically close to a promoter, where the concentration of RNA polymerase is high, the enhancer might get transcribed by accident.

Sometimes a number of enhancers are clustered together to form a **super-enhancer**. Super-enhancers are usually occupied by a high number of transcription factors and are able to stimulate higher levels of transcription than are regular enhancers. Examination of the chromatin surrounding super-enhancers suggests that the enhancers within the super-enhancer are in close physical proximity to one another and to the promoter of the gene they regulate. Super-enhancers appear to be important in determining cell identity.

Other sequences, called *silencers*, have an inhibitory effect on the transcription of distant genes. Like enhancers, silencers are position and orientation independent, and they contain binding sites for transcription factors that decrease transcription.

Insulators and Regulatory Neighborhoods

Most enhancers are capable of stimulating any promoter in their vicinity. Their effects are limited, however, by **insulators** (also called *boundary elements*), which are DNA sequences that block the effects of enhancers in a position-dependent manner. If an insulator lies between an enhancer and a promoter, it blocks the action of the enhancer, but if an insulator lies outside the region between the two, it has no effect (**Figure 17.8**). Specific proteins bind to insulators and play a role in their blocking activity.



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17.8 An insulator blocks the action of an enhancer on a promoter when the insulator lies between the enhancer and the promoter.

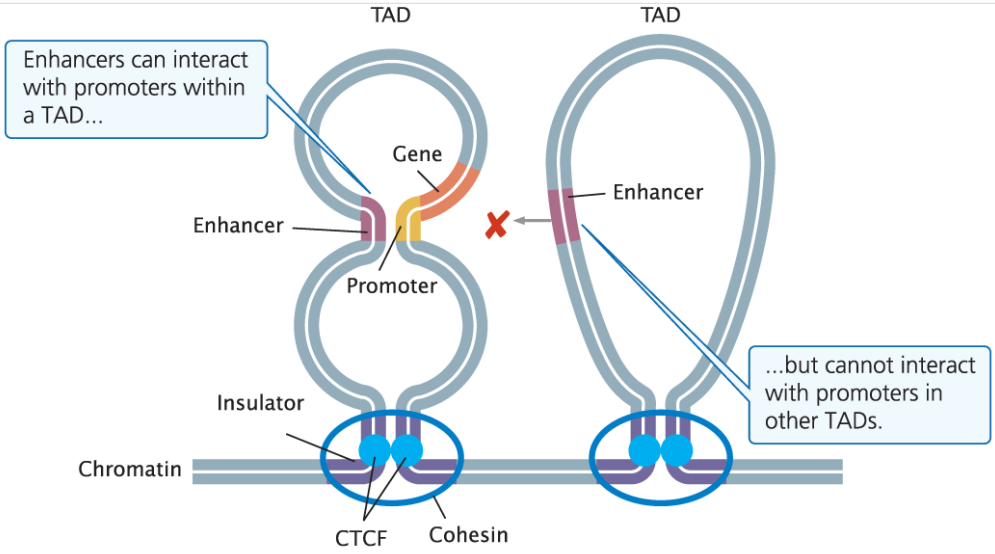
Description

The illustration shows various regions on a D N A fragment. The following regions are present from left to right: gene big A, transcription start site represented by an arrow to the left side below the bottom strand, gene big A's promoter, enhancer 1, insulator, enhancer 2, promoter for gene big B, transcription start site represented by an arrow to the right side above the top strand, gene big B. There is an insulator binding protein attached to the insulator protein-binding site. An arrow emerges from the enhancer 1 and points to gene big A's promoter. Another arrow from enhancer 1 points to insulator and has a cross mark over it. A caption reads, Enhancer 1 can stimulate the transcription of gene big A, but its effect on gene big B is blocked by the insulator. An arrow emerges from the enhancer 2 and points to gene big B's promoter. Another arrow from enhancer 2 points to insulator and has a cross mark over it. A caption reads, Enhancer 2 can stimulate the transcription of gene big B, but its effect on gene big A is blocked by the insulator.

Much recent research indicates that specific chromosome structures play an important role in eukaryotic gene regulation and that insulators may be involved in the formation of these structures. Insulators bind proteins that allow chromatin to form topologically associated domains (TADs), which were discussed in Chapter 11. Recall that TADs are large, spatially interacting regions of chromatin that are separated physically from other TADs. One model of how insulators work is that they bind proteins that cause chromatin loops, allowing enhancers to interact with promoters within the same TAD but preventing enhancers from interacting with promoters in different TADs. For example, a common insulator-binding protein in vertebrates is CTCF, which is found bound to DNA at the base of TADs and, with the help of cohesin, is thought to be involved in the formation of chromatin loops (**Figure 17.9**). In this way, insulators and their binding proteins may help create "neighborhoods" of regulatory elements and genes that are able to

physically interact but are insulated from regulatory elements in other neighborhoods. Some insulators also limit the spread of changes in chromatin structure that affect transcription. **› TRY PROBLEM 24**

THINK-PAIR-SHARE Question 5



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17.9 Insulators help create neighborhoods of gene regulation. One model suggests that insulators bind proteins that cause chromatin loops, allowing enhancers to interact with promoters within the same topologically associated domain (TAD) but preventing interactions with enhancers from different TADs.

Description

The illustration shows a double stranded chromatin with two topologically associated domain (T A D). The first T A D on the left has two loops. The point where the double strand extends upward to form the domain has a C T C F molecule, an enhancer at the beginning of the first loop, gene and promoter at the end of second loop, and a cohesin molecule where the first loop has to again straighten. The enhancer and promoter are close to each other. A caption reads, Enhancers can interact with promoters within a T A

D. The second T A D on the right has a single, loop. The C T C F molecule is present at the beginning of the loop, enhancer on the left part, and the cohesin molecule on the end of the loop. A caption extending from the enhancer reads, ellipses but cannot interact with promoters in other T A D‘s.

CONCEPTS

Some transcriptional regulator proteins bind to enhancers, which are regulatory elements that are distant from the gene whose transcription they stimulate. Insulators are DNA sequences that block the action of enhancers. Insulators may function by causing loops of chromatin that form interacting regions of genes and regulatory elements.

✔ CONCEPT CHECK 3

How does the binding of transcription factors to enhancers affect transcription at genes that are thousands of base pairs away?

Regulation of Transcriptional Stalling and Elongation

As we’ve seen, transcription in eukaryotes is often regulated through factors that affect the initiation of transcription, including changes in chromatin structure and transcription factors. Transcription may also be controlled through factors that affect stalling and elongation by RNA polymerase after transcription has been initiated.

The basal transcription apparatus—consisting of RNA polymerase, general transcription factors, and other proteins—assembles at the core promoter. When the initiation of transcription has taken place, RNA polymerase moves downstream, transcribing the structural gene and producing an RNA product. At some genes, RNA polymerase initiates transcription and transcribes 24 to 50 nucleotides of RNA but then pauses or stalls. For example, stalling is observed at genes that encode **heat-shock proteins** in *Drosophila*—proteins that help prevent damage by stressors such as extreme heat. Heat-shock proteins are produced by a large number of different genes. During times of environmental stress, transcription of all the heat-shock genes is greatly elevated. In the absence of stress, RNA polymerase initiates transcription at heat-shock genes in *Drosophila* but stalls downstream of the transcription start site. Stalled polymerases are released when stress is encountered, allowing rapid transcription of the genes and the production of heat-shock proteins that facilitate adaptation to the stressful environment.

Stalling was formerly thought to take place at only a small number of genes, but research now indicates that stalling is widespread throughout eukaryotic genomes, occurring at 30% to 50% of genes. Several factors that promote stalling have been identified. One of these factors is a protein called negative elongation factor (NELF), which binds to RNA polymerase and causes it to stall after initiation. Another protein, called positive transcription elongation factor b (P-TEFb), relieves stalling and promotes elongation by phosphorylating

NELF and RNA polymerase, perhaps by causing NELF to dissociate from the polymerase.

CONCEPTS

At some genes, RNA polymerase may pause or stall downstream of the promoter. Regulatory factors affect stalling and the elongation of transcription.

Coordinated Gene Regulation

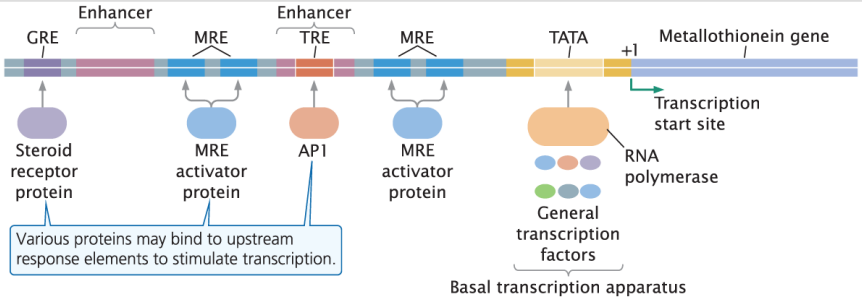
Although most eukaryotic cells do not possess operons, several eukaryotic genes may be activated by the same stimulus. Groups of bacterial genes are often coordinately expressed (turned on and off together) because they are physically clustered in an operon and share a promoter, but coordinately expressed genes in eukaryotic cells are not clustered. How, then, is the transcription of eukaryotic genes coordinated?

Genes that are coordinately expressed in eukaryotic cells are able to respond to the same stimulus because they share short regulatory sequences in their promoters or enhancers. For example, different eukaryotic heat-shock genes (see p. 511) possess a common regulatory sequence upstream of their transcription start sites. Such regulatory sequences are called **response elements**; they are short stretches of DNA that typically contain the same consensus

sequences at varying distances from the genes being regulated. The response elements are binding sites for transcription factors, which bind to the response elements and elevate transcription. If the same response element is present at multiple genes, it allows all those genes to be activated by the same stimulus. For example, transcription factors that bind to heat-shock elements activate genes that help cells respond to heat and other types of stress.

A single eukaryotic gene may be regulated by several different response elements. For example, the metallothionein gene encodes a protein that protects cells from the toxicity of heavy metals by binding to those metals and removing them from cells. Under normal conditions, the basal transcription apparatus assembles around the TATA box just upstream of the transcription start site for the metallothionein gene, but the apparatus alone is capable of only low rates of transcription. Other response elements found upstream of the metallothionein gene contribute to increasing its rate of transcription. For example, several copies of a metal response element (MRE) lie upstream of the metallothionein gene (**Figure 17.10**). The presence of heavy metals stimulates the binding of transcription factors to the MRE, which elevates the rate of transcription of the metallothionein gene. Because there are multiple copies of the MRE, high rates of transcription are induced by metals. Two enhancers are also located in the region upstream of the metallothionein gene. One of these enhancers contains a response element known as TRE, which stimulates transcription in the presence of a protein called AP1. A third response element,

called GRE, is located approximately 250 nucleotides upstream of the metallothionein gene and stimulates transcription in response to certain hormones.



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17.10 Multiple response elements are found in the region upstream of the metallothionein gene. The basal transcription apparatus assembles near the TATA box in the gene's promoter. In response to the presence of heavy metals, transcription factors bind to several metal response elements (MREs) and stimulate transcription. Another response element, TRE, is the binding site for transcription factor AP1, which is stimulated by phorbol esters. In response to glucocorticoid hormones, steroid receptor proteins bind to a third response element, GRE, located approximately 250 nucleotides upstream of the metallothionein gene, and stimulate transcription.

Description

The illustration shows a D N A double strand. The following regions with their corresponding proteins, from left to right, are as follows: G R E for steroid receptor protein; enhancer; two M R E regions for M R E activator protein; a T R E element within an enhancer for A P 1 protein; two M R E regions for M R E activator protein; T A T A box for a large R N A polymerase and six smaller general transcription factors that are collectively termed basal transcription apparatus; transcription start site represented by an arrow to the right side below the bottom strand; metallothionein gene. A caption reads, Various proteins may bind to upstream response elements to stimulate transcription.

This example illustrates a common feature of eukaryotic transcriptional control: a single gene may be activated by several different response elements found in both promoters and enhancers. Multiple response elements allow the same gene to be activated by different stimuli. At the same time, the presence of the same response element in different genes allows a single stimulus to activate multiple genes. In this way, response elements allow complex biochemical responses in eukaryotic cells.

17.4 Eukaryotic Genes Are Regulated by RNA Processing and Degradation

In bacteria, transcription and translation take place simultaneously. In eukaryotes, transcription takes place in the nucleus, and the pre-mRNAs then undergo processing before being moved to the cytoplasm for translation, which allows opportunities for gene control after transcription. Consequently, posttranscriptional gene regulation assumes an important role in eukaryotic cells. Gene regulation in eukaryotes often takes place during RNA processing and degradation.

Gene Regulation Through RNA Splicing

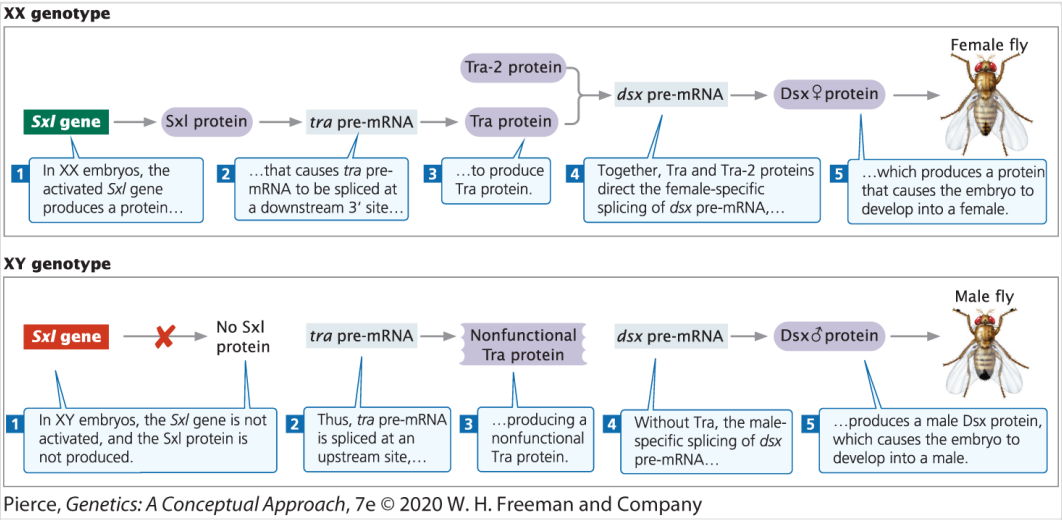
Alternative splicing allows pre-mRNA to be spliced in multiple ways, generating different proteins in different tissues or at different times in development (see Chapter 14). Many eukaryotic genes undergo alternative splicing; for example, it is estimated that 95% of all human genes with multiple exons are alternatively spliced. Thus, the regulation of splicing is an important means of controlling gene expression in eukaryotic cells.

As we saw in Section 14.2, proper splicing is dependent on the presence of consensus sequences at the 5′ splice site, the 3′ splice site, and the branch point. These consensus sequences determine the precise locations of introns and exons. Additional sequences called *exonic/intronic splicing enhancers* and *splicing silencers* help promote or repress the use of particular splice sites during the process of RNA splicing, resulting in alternative splicing outcomes. Proteins and ribonucleoprotein particles bind to these sequences and promote or repress splice-site selection. One group of proteins involved in splice-site selection consists of the **SR** (serine- and arginine-rich) **proteins**, which have two protein domains: one domain is an RNA-binding region and the other contains alternating serine and arginine amino acids. The precise mechanism by which SR proteins influence the choice of splice sites is poorly understood. One model suggests that SR proteins bind to splicing enhancers on the pre-mRNA and stimulate the attachment of small nuclear ribonucleoproteins (snRNPs), which then commit the site to splicing.

ALTERNATIVE SPLICING IN *DROSOPHILA* SEXUAL DEVELOPMENT

An example of gene regulation by alternative mRNA splicing is sex determination in fruit flies. Sex differentiation in *Drosophila* arises from a cascade of gene regulation. When two X chromosomes are present, a female-specific promoter is activated early in

development and stimulates the transcription of the *sex-lethal* (*Sxl*) gene (Figure 17.11). The protein encoded by *Sxl* regulates the splicing of the pre-mRNA transcribed from another gene called *transformer* (*tra*). The splicing pattern of *tra* pre-mRNA results in the production of the Tra protein (see Figure 17.11). Together with another protein (Tra-2), Tra stimulates the female-specific splicing of pre-mRNA from yet another gene called *doublesex* (*dsx*). This event produces a female-specific Dsx protein, which causes the embryo to develop female characteristics.



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17.11 Alternative splicing controls sex determination in *Drosophila*.

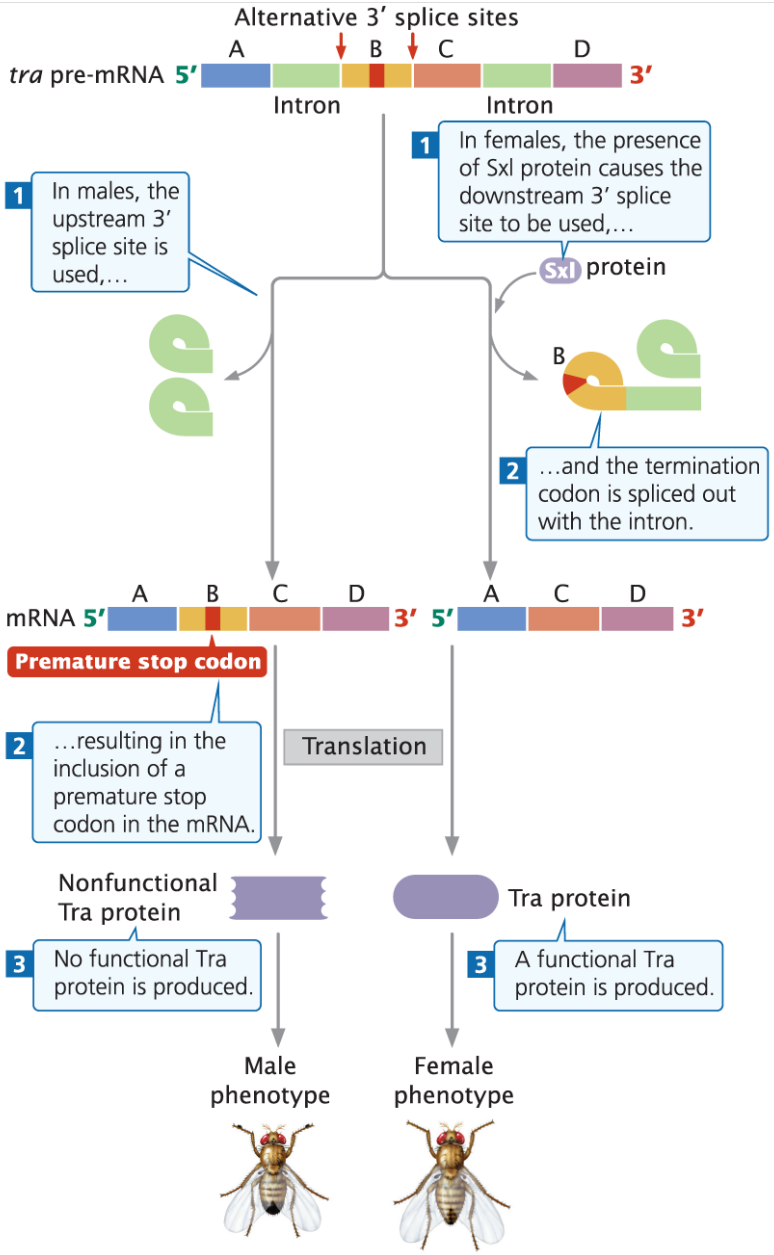
Description

Text at the top reads, X X genotype. The illustration shows big S small X small L gene on the left. A caption reads, In X X embryos, the activated big S small X small L gene produces a protein. The gene leads to big S small X small L protein formation on the right. The protein leads to t r a pre- m R N A formation on the right. A caption reads, that causes t r a pre m R N A to be spliced at a downstream 3 prime site. The m R N A produced leads to the formation of t r a protein on the right. A caption reads, to produce T r a protein. T r a-2 protein is present above the T r a protein. Together, t r a-2 and t r a protein lead to the

formation of d s x pre m R N A on the right. A caption reads, Together, T r a and T r a-2 proteins direct the female-specific splicing of d s x pre-m R N A. The d s x pre m R N A leads to formation of D s x female protein on the right. A caption reads, which produces a protein that causes the embryo to develop into a female. The female protein leads to development of a female fly on the right.

Text below reads, X Y genotype. The illustration shows big S small X small L gene on the left. A caption reads, In X Y embryos, the big S small X small L gene is not activated, and the big S small X small L protein is not produced. The gene is unable to produce the big S small X small L protein. A t r a pre-m R N A, on the right, leads to the production of nonfunctional t r a protein. A caption reads, Thus, t r a pre-m R N A is spliced at an upstream site, producing a nonfunctional T r a protein. A d s x pre-m R N A, on the right, leads to the formation of D s x male protein. A caption reads, Without T r a, the male specific splicing of d s x pre-m R N A, produces a male D s x protein, which causes the embryo to develop into a male. The male protein leads to the development of a male fly on the right.

In male embryos, which have a single X chromosome (see Figure 17.11), the promoter of the *Sxl* gene is inactive, so the gene is not transcribed, and no Sxl protein is produced. In the absence of Sxl protein, *tra* pre-mRNA is spliced at a different 3′ splice site to produce a nonfunctional form of Tra protein (**Figure 17.12**). In turn, the presence of this nonfunctional Tra in males causes *dsx* pre-mRNAs to be spliced differently from those in females, and a male-specific Dsx protein is produced (see Figure 17.11). This event causes the development of male-specific traits.



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17.12 Alternative splicing of *tra* pre-mRNA. Two alternative 3′ splice sites are present.

Description

The illustration shows a single stranded t r a pre-m R N A running from 5 prime to 3 prime. The pre-m-R N A going left to right is labeled as follows: exon A, intron, alternative 3 prime

splice site, exon B, alternative 3 prime splice site, exon C, intron, and exon D. Within exon B, there is a red vertical bar representing a premature stop codon. A downward arrow from this pre m R N A divides into two below.

On the left below, for males, the upstream 3 prime splice site is used. A caption reads, In males, the upstream 3 prime splice site is used. The introns are eliminated. The exons A, B, C, and D are arranged together, which results in the inclusion of a premature stop codon in the m-R N A. The m R N A runs from 5 prime to 3 prime. A caption reads, resulting in the inclusion of a premature stop codon in the m R N A. Translation occurs and a non functional t r a protein is produced below. A caption reads, No functional T r a protein is produced. The non functional t r a protein results in the development of the male fly below. Text below the fly reads, Male phenotype.

On the right below, for females, downstream 3 prime splice site is used. A big S small X small L protein is available. A caption reads, In females, the presence of big S small X small L protein causes the downstream 3 prime splice site to be used. The exon B along with the premature codon is spliced out with the introns. A caption reads, and the termination codon is spliced out with the intron. Exons A, C, and D are arranged together in the m R N A that runs from 5 prime to 3 prime. Translation occurs and a T r a protein is obtained below. A caption reads, A functional T r a protein is produced. The t r a protein results in the development of a female fly below. Text below the fly reads, Female phenotype.

In summary, the Tra, Tra-2, and Sxl proteins regulate alternative splicing patterns that produce male and female phenotypes in *Drosophila*. Exactly how these proteins regulate alternative splicing is not yet known, but the Sxl protein (produced only in females) might possibly block the upstream splice site on the *tra* pre-mRNA. This blockage would force the spliceosome to use the downstream 3′ splice site, which causes the production of Tra protein and eventually results in female traits (see Figure 17.12). **› TRY PROBLEM 25**

THINK-PAIR-SHARE Question 6

CONCEPTS

Eukaryotic genes can be regulated through the control of mRNA processing. The selection of alternative splice sites leads to the production of different proteins.

The Degradation of RNA

The amount of a protein that is synthesized depends on the amount of the corresponding mRNA that is available for translation. The amount of available mRNA, in turn, depends on both the rate of mRNA synthesis and the rate of mRNA degradation. Eukaryotic mRNAs are generally more stable than bacterial mRNAs, which typically last only a few minutes before being degraded. Nonetheless, there is great variation in the stability of eukaryotic mRNAs: some persist for only a few minutes, whereas others last for hours, days, or even months. These variations can produce large differences in the amount of protein that is synthesized.

Cellular RNA is degraded by ribonucleases, enzymes that specifically break down RNA. Most eukaryotic cells contain 10 or more types of ribonucleases, and there are several different pathways of mRNA degradation. In one pathway, the 5′ cap is first removed, followed by

$5' \rightarrow 3'$ removal of nucleotides. A second pathway begins at the $3'$ end of the mRNA and removes nucleotides in the $3' \rightarrow 5'$ direction. In a third pathway, the mRNA is cleaved at internal sites.

Messenger RNA degradation from the $5'$ end is most common and begins with the removal of the $5'$ cap. This pathway is usually preceded by the shortening of the poly(A) tail. Poly(A)-binding proteins (PABPs) normally bind to the poly(A) tail and contribute to its stability-enhancing effect. The presence of these proteins at the $3'$ end of the mRNA protects the $5'$ cap. When the poly(A) tail has been shortened below a critical limit, the $5'$ cap is removed, and nucleases then degrade the mRNA by removing nucleotides from the $5'$ end. These observations suggest that the $5'$ cap and the $3'$ poly(A) tail of eukaryotic mRNA physically interact with each other, most likely by the poly(A) tail bending around so that the PABPs make contact with the $5'$ cap (see Figure 15.18).

Much of RNA degradation takes place in specialized complexes called P bodies. However, P bodies appear to be more than simply destruction sites for RNA. P bodies can temporarily store mRNA molecules, which may later be released and translated. Thus, P bodies help control the expression of genes by regulating which RNA molecules are degraded and which are sequestered for later release. RNA degradation facilitated by small interfering RNAs (siRNAs) may also take place within P bodies (see Section 17.5).

Other parts of eukaryotic mRNA, including sequences in the 5′ untranslated region (5′ UTR), the coding region, and the 3′ UTR, also affect mRNA stability. Some short-lived eukaryotic mRNAs have one or more copies of the consensus sequence 5′–AUUUAUAA–3′, referred to as the AU-rich element, in the 3′ UTR. Messenger RNAs containing AU-rich elements are degraded by a mechanism in which microRNAs take part (see Section 17.5).

CONCEPTS

The stability of mRNA influences gene expression by affecting the amount of mRNA available to be translated. The stability of mRNA is affected by the 5′ cap, the poly(A) tail, the 5′ UTR, the coding region, and sequences in the 3′ UTR.

✔ CONCEPT CHECK 4

How does the poly(A) tail affect mRNA stability?

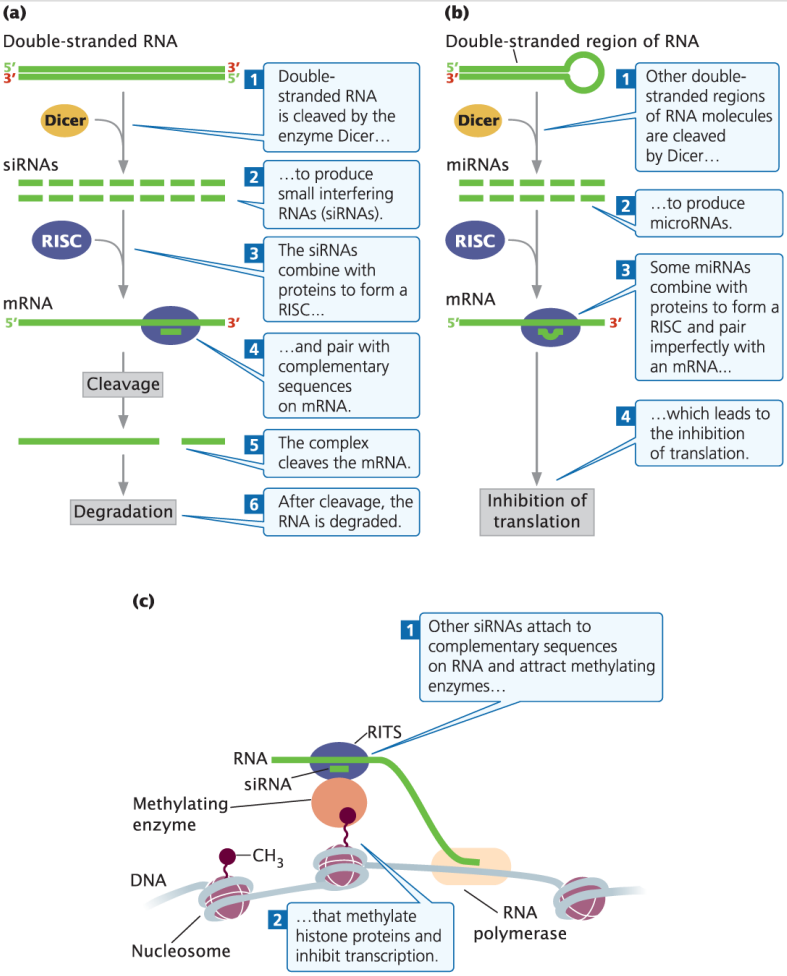
17.5 RNA Interference Is an Important Mechanism of Gene Regulation

The expression of a number of eukaryotic genes is controlled through RNA interference, also known as *RNA silencing* (see Chapter 14). Research suggests that as many as 30% of human genes are regulated by RNA interference. RNA interference is widespread in eukaryotes, existing in fungi, plants, and animals. This mechanism is also widely used as a technique for artificially regulating gene expression in genetically engineered organisms (see Chapter 19).

Small Interfering RNAs and MicroRNAs

RNA interference is triggered by very small RNA molecules known as microRNAs (miRNAs) and small interfering RNAs (siRNAs), depending on their origin and mode of action (see Chapter 14). An enzyme called Dicer cleaves and processes double-stranded RNA to produce single-stranded siRNAs or miRNAs 21 to 25 nucleotides long (**Figure 17.13**), which combine with proteins to form an RNA-induced silencing complex (RISC). The RNA component of RISC then pairs with complementary base sequences in specific mRNA molecules, most often with sequences in the 3′ UTR of the mRNA.

Small interfering RNAs tend to base pair perfectly with mRNAs, whereas microRNAs often form less-than-perfect pairings.



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17.13 RNA silencing leads to the degradation of mRNA or to the inhibition of translation or transcription. (a) Small interfering RNAs (siRNAs) degrade mRNA by cleavage. (b) MicroRNAs (miRNAs) lead to the inhibition of translation. (c) Some siRNAs bring about methylation of histone proteins, inhibiting transcription.

Description

Part a at the top left shows a double stranded R N A. The top strand runs from 5 prime to 3 prime and the bottom strand runs from 3 prime to 5 prime. A dicer molecule enters the process. A caption reads, Double stranded R N A is cleaved by the enzyme Dicer. It leads

to the R N A fragmentation thereby producing several s i R N A's. A caption reads, to produce small interfering R N A's (s i R N A's). The s i R N A's together with proteins form a R I S C complex. A caption reads, The s I R N A's combine with proteins to form a R I S C. The R I S C complex pairs with a complementary sequence on an m R N A. A caption reads, and pair with complementary sequences on m R N A. Cleavage occurs. The m R N A strand on which the R I S C complex was paired is cleaved into two parts. A caption reads, The complex cleaves the m R N A. This leads to degradation below. A caption reads, After cleavage, the R N A is degraded.

Part b, at the top right, shows a double stranded R N A with a loop on its right end. The top strand runs from 5 prime to 3 prime and the bottom strand runs from 3 prime to 5 prime. A dicer molecule enters the process. A caption reads, Other double stranded regions of R N A molecules are cleaved by Dicer. It leads to the R N A fragmentation thereby producing several m i R N A's. A caption reads, to produce Micro R N A. Some m i R N A's combine with proteins to form a R I S C complex. This R I S C complex pairs imperfectly on an m R N A. A caption reads, Some m i R N A's combine with proteins to form a R I S C and pair imperfectly with an m R N A, which leads to the inhibition of translation. Translation is inhibited.

Part c at the bottom center shows methylation of histone proteins by s i R N A's. A string of three nucleosomes with D N A wrapped around it is present below. A tiny wavy line, representing C H subscript 3, is extending out of the two nucleosomes. A methylating enzyme is bound to C H subscript 3 of the nucleosome in the center. One R I T S molecule sits above methylating enzyme and a lowercase s I R N A is present just above the methylating enzyme. An R N A polymerase is attached on the D N A between second and third nucleosome. An R N A extends from R N A polymerase to the R I T S molecule. A caption reads, Other s i R N A's attach to complementary sequences on R N A and attract methylating enzymes that methylate histone proteins and inhibit transcription.

Mechanisms of Gene Regulation by RNA Interference

Small interfering RNAs and microRNAs regulate gene expression through at least four distinct mechanisms: (1) cleavage of mRNA, (2) inhibition of translation, (3) transcriptional silencing, and (4) degradation of mRNA.

RNA CLEAVAGE

RISCs that contain an siRNA (and some that contain an miRNA) pair with an mRNA molecule and cleave the mRNA near the middle of the bound siRNA (see Figure 17.13a). This cleavage is carried out by a protein that is sometimes referred to as Slicer. After cleavage, the mRNA is further degraded. Thus, the presence of siRNAs and miRNAs increases the rate at which mRNAs are broken down and decreases the amount of protein produced.

INHIBITION OF TRANSLATION

Some miRNAs regulate genes by inhibiting the translation of complementary mRNAs (see Figure 17.13b). For example, an important gene in flower development in *A. thaliana* is *APETALA2*. The expression of this gene is regulated by an miRNA that base pairs with nucleotides in the coding region of *APETALA2* mRNA and inhibits its translation.

The exact mechanism by which miRNAs repress translation is still poorly understood, but some research suggests that they can inhibit

the initiation step of translation as well as steps after initiation, such as ribosome stalling or premature termination. Many mRNAs have multiple miRNA-binding sites, and translation is most efficiently inhibited when multiple miRNAs are bound to the mRNA.

TRANSCRIPTIONAL SILENCING

Some siRNAs silence transcription by altering chromatin structure. These siRNAs combine with proteins to form a complex called RITS (for *R*NA-*i*nduced *t*ranscriptional *s*ilencing; see Figure 17.13c), which is analogous to RISC. The siRNA component of RITS binds to a complementary sequence in an RNA molecule that is in the process of being transcribed. The RITS complex then represses transcription by attracting enzymes that methylate the tails of histone proteins. The addition of methyl groups to the histones causes them to bind DNA more tightly, restricting the access of the proteins and enzymes necessary to carry out transcription (see Histone Modification in Section 17.2).

SLICER-INDEPENDENT DEGRADATION OF mRNA

A final mechanism by which miRNAs regulate gene expression is by triggering the decay of mRNA through a process that does not require Slicer activity. This mechanism plays a role in the degradation of short-lived mRNAs that contain an AU-rich element in their 3′ UTR. AU-rich elements are found, for example, in mRNAs

that encode cytokines, proteins that play an important role in the body's response to infection and injury. Overproduction of cytokines, or their production at the wrong time, can lead to shock and autoimmune disease, so precise regulation of cytokine synthesis is critical. Researchers have identified an miRNA with a sequence that is complementary to the AU-rich element. This miRNA binds to the AU-rich element and, in a way that is not yet fully understood, brings about the degradation of the mRNA in a process that requires Dicer and RISC. › **TRY PROBLEM 27**

The Control of Development by RNA Interference

Much of development in multicellular eukaryotes is controlled through gene regulation: different genes are turned on and off at specific times (see Chapter 22). In fact, when miRNAs were first discovered, researchers noticed that a mutation in an miRNA in *C. elegans* caused a developmental defect. Research has demonstrated that miRNA molecules are key factors in controlling development in animals, including humans, and plants. For example, the vertebrate heart develops through the programmed differentiation and proliferation of cardiac muscle cells, which are controlled by a specific miRNA termed miR-1-1.

Through their effects on gene expression, miRNAs play important roles in many diseases and disorders. For example, a genetic form of hearing loss has been associated with a mutation in the gene that encodes an miRNA. Other miRNAs are associated with heart disease. One miRNA, called miR-1-2, is highly expressed in heart muscle. Mice genetically engineered to express only 50% of the normal amount of miR-1-2 frequently have holes in the wall that separates the left and right ventricles, a common congenital heart defect seen in newborn humans. Overexpression of another miRNA, called miR-1, in the hearts of adult mice causes cardiac arrhythmia—irregular electrical activity of the heart that can be life-threatening in humans. And finally, numerous studies have demonstrated that abnormal expression of miRNAs plays a role in many cancers; miRNAs are now being used for cancer prognosis and experimental treatment.

RNA Crosstalk

An additional layer of gene regulation—called RNA crosstalk—arises from interactions and competition among miRNAs, mRNAs, and other RNA molecules. These interactions occur because different RNA molecules often share binding sites for the same miRNA. If the miRNA is in limited supply, then the RNAs with shared miRNA binding sites compete with one another for the limited copies of the miRNA.

The RNA molecules that compete for miRNAs include some noncoding RNAs. In Chapter 14, we discussed long noncoding RNAs (lncRNAs), which are RNA molecules over 200 nucleotides long that do not encode a protein. Some lncRNAs have multiple binding sites for miRNAs that are shared with mRNAs. The lncRNAs serve as molecular decoys, attracting the miRNAs and tying them up so that they are not available to bind to mRNA. Some recently discovered circular RNAs (see Chapter 14) also have multiple binding sites for miRNAs and similarly serve as decoys for miRNAs.

The same type of competition can occur among RNA molecules that share binding sites for proteins that function in RNA splicing, stability, and translation. RNA molecules that engage in this type of crosstalk are collectively referred to as competing endogenous RNAs (ceRNAs). Numerous examples of ceRNAs have been discovered, and RNA crosstalk may be an important component of gene regulation.

CONCEPTS

RNA interference is initiated by double-stranded RNA molecules that are cleaved and processed. The resulting siRNAs or miRNAs combine with proteins to form complexes that bind to complementary sequences in mRNA or DNA. The siRNAs and miRNAs affect gene expression by cleaving mRNA, inhibiting translation, altering chromatin structure, or triggering RNA degradation. Different RNA molecules that share binding sites for miRNAs may compete among themselves for available miRNAs, creating an additional layer of gene regulation known as RNA crosstalk.

✔ CONCEPT CHECK 5

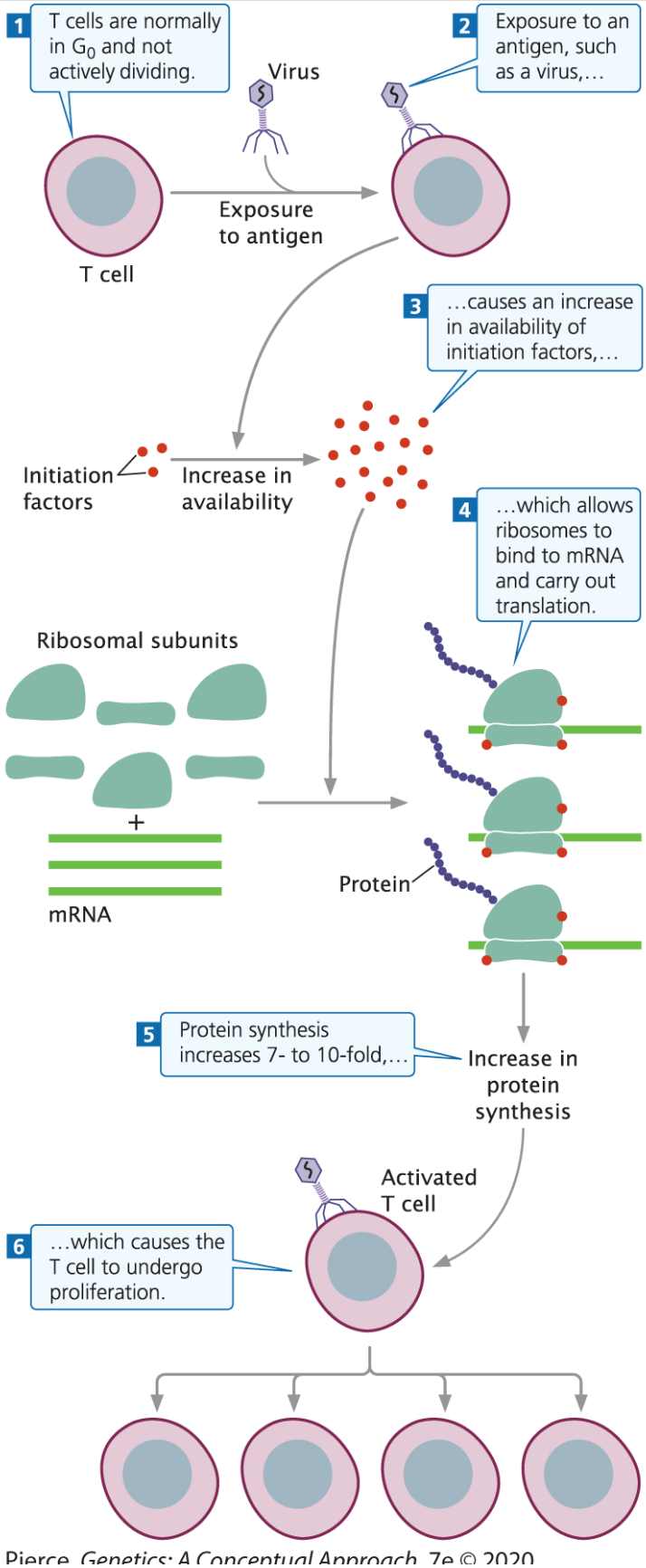
In RNA silencing, siRNAs and miRNAs usually bind to which part of the mRNA molecules that they control?

a. $5'$ UTR
b. Coding region
c. $3'$ poly(A) tail
d. $3'$ UTR

17.6 The Expression of Some Genes Is Regulated by Processes That Affect Translation or by Modifications of Proteins

Ribosomes, charged tRNAs, initiation factors, and elongation factors are all required for the translation of mRNA molecules (see Chapter 15). The availability of these components affects the rate of translation and therefore influences gene expression. For example, the activation of T lymphocytes (T cells) is critical to the development of immune responses to viruses (see Chapter 22). T cells are normally in the G_0 stage of the cell cycle and not actively dividing. Upon exposure to viral antigens, however, specific T cells become activated and undergo rapid proliferation (**Figure 17.14**). Activation begins with a 7- to 10-fold increase in protein synthesis that causes the cells to enter the cell cycle and proliferate. This global burst of protein synthesis does not require an increase in mRNA synthesis. Instead, it is due to the increased availability of initiation factors, which allow ribosomes to bind to mRNA and begin translation. This increase in initiation factors leads to more translation of the existing mRNA molecules, increasing the overall amount of protein synthesized. In a similar way, insulin stimulates protein synthesis by increasing the availability of initiation factors. Initiation factors exist in inactive forms and, in response to various

cell signals, can be activated by chemical modifications of their structure, such as phosphorylation.



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17.14 The expression of some eukaryotic genes is regulated by the availability of components required for translation. In this example, exposure to an antigen stimulates increased availability of initiation factors and a subsequent increase in protein synthesis, leading to T-cell proliferation.

Description

The illustration shows a round T cell, at the top left. A caption reads, T cells are normally in G subscript 0 and not actively dividing. The T cell is exposed to a virus. Text reads, exposure to antigen. The virus is present at the top of T cell. A caption reads, Exposure to an antigen, such as a virus. Few initiation factors are present below. T cell with virus leads to increase in availability of these initiation factors. A large number of initiation factors are obtained on the right. Three dissociated ribosomal units are shown below along with three m R N A's. The availability of large number of initiation factors causes the ribosomal units to bind the m R N A's. Each m R N A has a ribosome attached to it. A chain of protein is emerging from the ribosomes. Initiation factors are also attached to these ribosomes. A caption reads, which allows ribosomes to bind to m R N A and carry out translation. This leads to increase in protein synthesis. A caption reads, Protein synthesis increases 7- to 10-fold. This further leads to an activated T cell. Finally, T cell undergoes proliferation. Four cells are obtained below. A caption reads, which causes the T cell to undergo proliferation.

In addition to increasing overall protein synthesis, translational control can also target the synthesis of specific proteins. For example, research has demonstrated that eukaryotic initiation factor 3 (eIF3), which functions generally to initiate eukaryotic translation, can also bind to secondary structures in the 5′ untranslated regions of specific mRNAs and either stimulate or repress their translation, depending on the particular sequence to which it binds. The initiation of translation in some mRNAs is regulated by proteins that bind to the 5′ UTR of certain mRNAs and inhibit the binding of

ribosomes, in a manner similar to the binding of repressor proteins to operators that prevents the transcription of structural genes in prokaryotes. The translation of other mRNAs is affected by the binding of proteins to sequences in the 3′ UTR.

As we saw in Section 17.3, the presence of extreme heat or other stressors induces the synthesis of heat-shock proteins, which reduce cellular damage resulting from those stressors. Transcription of heat-shock genes is rapidly increased by the release of stalled RNA polymerases when stress is encountered. Translational control also plays a role in the induction of heat-shock proteins. In response to heat shock, adenosine nucleotides in the 5′ UTRs of mRNAs transcribed from heat-shock genes are preferentially methylated to form N^6-methyladenosine. The presence of N^6-methyladenosine then promotes cap-independent initiation of translation (see p. 450 in Chapter 15), leading to the synthesis of heat-shock proteins.

Many eukaryotic proteins are extensively modified after translation by the selective cleavage and trimming of amino acids from the ends; by acetylation; or by the addition of phosphate groups, carboxyl groups, methyl groups, carbohydrates, or ubiquitin (a small protein) (see Chapter 15). Control of these modifications, which affect the transport, function, stability, and activity of the proteins, may also play a role in gene expression.

THINK-PAIR-SHARE Questions 7 and 8

CONCEPTS

The availability of ribosomes, charged tRNAs, and initiation and elongation factors may affect the rate of translation. Translation of some mRNAs is regulated by proteins that bind to the $5'$ and $3'$ untranslated regions of the mRNA.

CONNECTING CONCEPTS CONNECT

A Comparison of Bacterial and Eukaryotic Gene Control

Now that we have considered the major types of gene regulation in bacteria (in Chapter 16) and eukaryotes (in this chapter), let's consider some of the similarities and differences in bacterial and eukaryotic gene control.

1. Much of gene regulation in bacterial cells is at the level of transcription (although it does exist at other levels). Gene regulation in eukaryotic cells takes place at multiple levels, including chromatin structure, transcription, mRNA processing, translation, RNA stability, and posttranslational modification of proteins.
2. Complex biochemical and developmental events in bacterial and eukaryotic cells may require a cascade of gene regulation, in which the activation of one set of genes stimulates the activation of another set.
3. Much of gene regulation in both bacterial and eukaryotic cells is accomplished through proteins that bind to specific sequences in DNA. Regulatory proteins come in a variety of types, but most can be characterized according to a small set of DNA-binding motifs.
4. Chromatin structure plays a role in eukaryotic (but not bacterial) gene regulation. In general, condensed chromatin represses gene expression; chromatin structure must therefore be altered before transcription can take place. Chromatin structure is altered by chromatin-remodeling complexes, modification of histone proteins, and DNA methylation.
5. Modifications to chromatin structure in eukaryotes may lead to epigenetic changes, which are changes that affect gene expression and are passed on to other cells or future generations.
6. In bacterial cells, many genes are clustered in operons and are coordinately expressed by transcription into a single mRNA molecule. In contrast, many eukaryotic genes have their own promoters. Coordinated gene regulation in eukaryotic cells often takes place through common response elements located in the promoters and enhancers of genes.

Different genes that share the same response element are influenced by the same regulatory protein.

7. Regulator proteins that affect transcription exhibit two basic types of control: *repressors* inhibit transcription (negative control) and *activators* stimulate transcription (positive control). Both negative control and positive control are found in bacterial and eukaryotic cells.
8. The initiation of transcription is a relatively simple process in bacterial cells, and regulator proteins function by blocking or stimulating the binding of RNA polymerase to DNA. In contrast, eukaryotic transcription requires complex machinery that includes RNA polymerase, general transcription factors, and other transcription factors, allowing transcription to be influenced by multiple factors.
9. Some eukaryotic transcription factors function at a distance from the gene by binding to enhancers, causing the formation of a loop in the DNA, which brings the promoter and enhancer into close proximity. Some sequences analogous to enhancers that act on distant genes have been described in bacterial cells, but they appear to be less common.
10. The greater time lag between transcription and translation in eukaryotic cells than in bacterial cells allows mRNA stability and mRNA processing to play larger roles in eukaryotic gene regulation.
11. Complementary RNA molecules (antisense RNA) may act as regulators of gene expression in bacteria. Regulation by siRNAs and miRNAs, which is extensive in eukaryotes, is absent in bacterial cells.

These similarities and differences in gene regulation in bacteria and eukaryotes are summarized in **Table 17.1**.

TABLE 17.1 Comparison of gene control in bacteria and eukaryotes

Characteristic	Bacterial Gene Control	Eukaryotic Gene Control
Levels of regulation	Primarily transcription	Many levels
Cascades of gene	Present	Present

regulation		
DNA-binding proteins	Important	Important
Role of chromatin structure	Absent	Important
Presence of operons	Common	Uncommon
Negative and positive control	Present	Present
Initiation of transcription	Relatively simple	Relatively complex
Enhancers	Less common	More common
Transcription and translation	Occur simultaneously	Occur separately
Regulation by siRNAs and miRNAs	Absent	Common

Chapter 17 Review

CHAPTER SUMMARY

Section 17.1 Learning Objective: Be able to list some ways that gene regulation in bacterial and eukaryotic cells differ.

- Eukaryotic cells differ from bacteria in several ways that affect gene regulation, including in the absence of operons, the presence of chromatin, and the presence of a nuclear membrane.

Section 17.2 Learning Objective: Be able to describe modifications to chromatin structure that affect gene expression in eukaryotes.

- In eukaryotic cells, chromatin structure represses gene expression. In transcription, chromatin structure may be altered by chromatin-remodeling complexes that reposition nucleosomes.
- Chromatin structure is altered by modifications of histone proteins, including acetylation, phosphorylation, and methylation.
- The methylation of DNA in eukaryotes occurs primarily in the form of 5-methylcytosine at CpG nulceotides, which inhibits transcription.

Section 17.3 Learning Objective: Be able to describe different types of regulatory sequences that affect the initiation and continuation of transcription.

- The initiation of eukaryotic transcription is controlled by general transcription factors that are part of the basal transcription apparatus and by other transcription factors that stimulate or repress normal levels of transcription by binding to regulatory promoters and enhancers.
- Enhancers affect the transcription of distant genes. Transcription factors bind to enhancers and interact with the basal transcription apparatus by causing the intervening DNA to loop out.
- DNA sequences called insulators limit the action of enhancers by blocking their action in a position-dependent manner.
- Some regulatory factors cause RNA polymerase to stall downstream of the promoter.
- Coordinately controlled genes in eukaryotic cells respond to the same factors because they share response elements that are stimulated by the same transcription factor.

Section 17.4 Learning Objective: Be able to describe the role of RNA splicing and degradation in gene regulation.

- Gene expression in eukaryotic cells can be influenced by RNA splicing. The same pre-mRNA may be spliced to produce different proteins.
- Gene expression can be regulated by changes in RNA stability. The 5′ cap, the poly(A) tail, the 5′ UTR, the coding region, and

sequences in the 3′ UTR are important in controlling the stability of eukaryotic mRNAs.
- Proteins that bind to the 5′ and 3′ ends of eukaryotic mRNA can affect its translation.

Section 17.5 Learning Objective: Be able to describe how RNA interference brings about gene regulation.

- RNA interference plays an important role in eukaryotic gene regulation. Small RNA molecules (siRNAs and miRNAs) cleaved from double-stranded RNA combine with proteins and bind to complementary sequences on mRNA or DNA. These complexes cleave RNA, inhibit translation, silence transcription, or affect RNA degradation. RNA molecules with shared miRNA binding sites may compete for available copies of miRNAs, creating an added level of gene regulation.

Section 17.6 Learning Objective: Be able to describe how translation and modification of proteins affect gene expression.

- Control of the posttranslational modification of proteins may play a role in gene expression.
- Bacterial and eukaryotic gene regulation share some common features but also differ in significant ways.

WORKED PROBLEM

What would be the effect of a mutation that causes a poly(A)-binding protein to be nonfunctional?

» Solution Strategy

What information is required in your answer to the problem?

The effect of a mutation that eliminates the function of a poly(A)-binding protein.

What information is provided to solve the problem?

- A mutation occurs in a gene that encodes a poly(A)-binding protein (PABP).
- The mutation causes the PABP to be nonfunctional.

For help with this problem, review:

The Degradation of RNA in Section 17.4.

Addition of the Poly(A) Tail in Section 14.2.

» Solution Steps

Messenger RNA can be degraded from the $5'$ end, from the $3'$ end, or through internal cleavage. Degradation from the $5'$ end requires the removal of the $5'$ cap and is usually preceded by the shortening of

the poly(A) tail. Poly(A)-binding proteins bind to the poly(A) tail and prevent it from being shortened. Thus, the presence of these proteins on the poly(A) tail protects the 5′ cap, which prevents RNA degradation. If the gene for a poly(A)-binding protein were mutated in such a way that nonfunctional PABP was produced, the protein would not bind to the poly(A) tail of the mRNAs with which it interacts. The tail would be shortened prematurely, the 5′ cap removed, and the mRNA degraded more easily. The end result would be less mRNA and thus less protein synthesis.

Recall: The poly(A) tail affects the stability of mRNA.

IMPORTANT TERMS

DNase I hypersensitive site
chromatin-remodeling complex
histone code
CpG island
mediator
super-enhancer
insulator
heat-shock protein
response element
SR protein

ANSWERS TO CONCEPT CHECKS

1. Three of these processes are chromatin remodeling, the modification of histone proteins (e.g., methylation and acetylation of histones), and DNA methylation.
2. b
3. The DNA between the enhancer and the promoter loops out, so that transcription factors bound to the enhancer are able to interact directly with the basal transcription apparatus.
4. The poly(A) tail stabilizes the 5′ cap, which must be removed before the mRNA molecule can be degraded from the 5′ end.
5. d

Chapter 17 Assessment

COMPREHENSION QUESTIONS

Introduction

1. How similar are the genomes of humans and chimpanzees? What genetic changes might be responsible for the large differences in the anatomy, physiology, and behavior of humans and chimpanzees?

Section 17.2

2. Where are DNase I hypersensitivity sites found, and what do they indicate about the nature of chromatin?

3. What changes take place in chromatin structure, and what role do these changes play in eukaryotic gene regulation?

4. What is the histone code?

5. How is chromatin immunoprecipitation used to determine the locations of histone modifications in the genome?

Section 17.3

6. Briefly explain how transcription factors affect the level of transcription of eukaryotic genes.

7. What is an enhancer? How does it affect the transcription of distant genes?

8. What is an insulator?

9. What is a response element? How do response elements bring about the coordinated expression of eukaryotic genes?

Section 17.4

10. Outline the role of alternative splicing in the control of sex differentiation in *Drosophila*.

11. What role does RNA stability play in gene regulation? What controls RNA stability in eukaryotic cells?

Section 17.5

12. Briefly list some of the ways in which siRNAs and miRNAs regulate genes.

Section 17.6

13. How does bacterial gene regulation differ from eukaryotic gene regulation? How are they similar?

APPLICATION QUESTIONS AND PROBLEMS

Section 17.2

14. CONNECT DNase I hypersensitivity provides information about changes in chromatin structure (see Chapter 11). At which genes and states of development shown in **Figure 11.7** is chromatin in an open configuration?

15. DATA ANALYSIS Malaria, one of the most pervasive and destructive of all infectious diseases, is caused by protozoan parasites of the genus *Plasmodium*, which are transmitted from person to person by mosquitoes. *Plasmodium* parasites are able to evade the host immune system by constantly altering the expression of their *var* genes, which encode *Plasmodium* surface antigens (L. H. Freitas-Junior et al. 2005. *Cell* 121:25–36). Individual *var* genes are expressed when chromatin structure is disrupted by chemical changes in histone proteins. What type of chemical changes in the histone proteins might be responsible for these changes in gene expression?

16. A geneticist is trying to determine how many genes are found in a 300,000-bp region of DNA. Analysis shows that four different areas within the 300,000-bp region have H3K4me3

modifications. What might their presence suggest about the number of genes located there?

17. In a line of human cells grown in culture, a geneticist isolates a temperature-sensitive mutation at a locus that encodes an acetyltransferase enzyme; at temperatures above 38°C, the mutant cells produce a nonfunctional form of the enzyme. What would be the most likely effect of this mutation if the cells were grown at 40°C?

18. CONNECT How is the histone code different from the genetic code discussed in Chapter 15 (see **Figure 15.10**)?

19. *What would be the most likely effect of deleting *flowering locus D* (*FLD*) in *A. thaliana*?

20. *X31b is an experimental compound that is taken up by rapidly dividing cells. Research has shown that X31b stimulates the methylation of DNA. Some cancer researchers are interested in testing X31b as a possible drug for treating prostate cancer. Offer a possible explanation for why X31b might be an effective anticancer drug.

Section 17.3

21. CONNECT How do repressors that bind to silencers in eukaryotes differ from repressors that bind to operators in bacteria? (See Chapter 16 for information on repressors in bacteria.)

22. Examine Figure 17.7. What would be the effect on transcription if a mutation occurred in the gene that encodes GAL3, so that no functional GAL3 was produced?

23. What would be the effect of moving the insulator shown in Figure 17.8 to a position between enhancer II and the promoter for gene B?

24. *An enhancer is surrounded by four genes (*A*, *B*, *C*, and *D*), as shown in the accompanying diagram. An insulator lies between gene *C* and gene *D*. On the basis of the positions of the genes, the enhancer, and the insulator, the transcription of which genes is most likely to be stimulated by the enhancer? Explain your reasoning.

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Description
The illustration shows a double stranded D N A fragment. The regions labeled, from left to right, are as follows: gene big A, gene big B, enhancer, gene big C, insulator, and gene big D.

Section 17.4

25. *What will be the effect on sexual development in newly fertilized *Drosophila* embryos if the following genes are deleted?
    a. *sex-lethal*
    b. *transformer*

c. *doublesex*

26. CONNECT Examine **Figure 17.12**. What would be the effect of a mutation that eliminated the downstream 3′ splice site at the end of exon B in the *tra* pre-mRNA? (See Chapter 14 for a review of RNA splicing.)

27. *Some eukaryotic mRNAs have an AU-rich element in the 3′ untranslated region. What would be the effect on gene expression if this element were mutated or deleted?

28. A strain of *A. thaliana* possesses a mutation in the *APETALA2* gene. As a result of this mutation, much of the 3′ UTR of the mRNA transcribed from the gene is deleted. What is the most likely effect of this mutation on the expression of the *APETALA2* gene?

Section 17.5

29. Suppose a geneticist introduced a small interfering RNA (siRNA) that was complementary to the *FLC* mRNA in **Figure 17.3**. What would be the effect on flowering of *Arabidopsis*? Explain your answer.

CHALLENGE QUESTIONS

Section 17.3

30. The yeast gene *SER3*, whose product has a role in serine biosynthesis, is repressed during growth in a nutrient-rich medium, so little transcription takes place, and little SER3 enzyme is produced under these conditions. In an investigation of the repression of the *SER3* gene, a region of DNA upstream of *SER3* was found to be heavily transcribed when *SER3* is repressed (J. A. Martens, L. Laprade, and F. Winston. 2004. *Nature* 429:571–574). Within this upstream region is a promoter that stimulates the transcription of an RNA molecule called *SRG1* RNA (for *SER3 r*egulatory *g*ene 1). This RNA molecule has none of the sequences necessary for translation. Mutations in the promoter for *SRG1* result in the disappearance of *SRG1* RNA, and these mutations remove the repression of *SER3*. When RNA polymerase binds to the *SRG1* promoter, the polymerase travels downstream, transcribing the *SRG1* RNA, and passes through and transcribes the promoter for *SER3*. This activity leads to the repression of *SER3*. Propose a possible explanation for how the transcription of *SRG1* might repress the transcription of *SER3*. (Hint: Remember that the *SRG1* RNA does not encode a protein.)

Section 17.5

31. A common feature of many eukaryotic mRNAs is the presence of a rather long 3′ UTR, which often contains consensus sequences. Creatine kinase B (CK-B) is an important enzyme in cellular metabolism. Certain cells—termed U937D cells—have

lots of CK-B mRNA, but no CK-B enzyme is present. In these cells, the 5′ end of the CK-B mRNA is bound to ribosomes, but the mRNA is apparently not translated. Something inhibits the translation of the CK-B mRNA in these cells.

Researchers introduced numerous short segments of RNA containing only 3′ UTR sequences into U937D cells. As a result, the U937D cells began to synthesize the CK-B enzyme, but the total amount of CK-B mRNA did not increase. The introduction of short segments of other RNA sequences did not stimulate the synthesis of CK-B; only the 3′ UTR sequences turned on the translation of the enzyme.

On the basis of these results, propose a mechanism for the inhibition of CK-B translation in the U937D cells. Explain how the introduction of short segments of RNA containing the 3′ UTR sequences might remove that inhibition.

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 17.2

1. Recent research has shown that activation of a topoisomerase enzyme (see Chapter 11) leads to greater expression of some genes in neurons. These genes remain silent until

topoisomerase is activated; then they are rapidly transcribed. Propose a mechanism for how topoisomerase might stimulate gene expression.

2. Mutations in genes encoding chromatin-remodeling complexes have been identified in high frequencies in some human cancers. For example, mutations in components of the human SWI–SNF remodeling complex were found in 19% of tumors in a group of cancers. How might a mutation in a chromatin-remodeling complex contribute to cancer?

Section 17.3

3. What do you think would be the overall effect of reducing the amount of mediator present in a cell?

4. Some DNA nucleotides are located within the coding regions of genes, where they specify the amino acid sequence of a protein. Other nucleotides are found in regulatory elements, where they serve as binding sites for regulatory proteins, such as transcription factors. It has long been assumed that sequences within coding regions and sequences within regulatory elements are independent, but recent research has determined that about 15% of human codons—dubbed duons—serve both to encode amino acids and as binding sites for regulatory proteins. How might the presence of duons affect which synonymous codons are used in the genetic code?

5. Mammals are anatomically and physiologically more complex than roundworms, yet both organisms have approximately the

same number of genes, about 20,000. Some biologists have argued that mammals and other vertebrates have evolved increased complexity by means of pleiotropy—in which each gene encodes multiple characteristics—and that pleiotropy was made possible by the evolution of additional enhancers. Propose an explanation for how additional enhancers might produce increased pleiotropy.

Section 17.4

6. Research has demonstrated that the composition of a gene's promoter can affect alternative splicing of its RNA transcript. For example, promoters that are activated by certain transcription factors cause one form of alternative splicing; when these factors are not present, a different form of splicing takes place. Propose some possible mechanisms by which the promoter could affect alternative splicing.

Section 17.6

7. As discussed in Chapter 15, some eukaryotic mRNAs have internal ribosome binding sites, where ribosomes can bind without first attaching to the 5′ cap. Yet all eukaryotic mRNAs are capped, and cap-dependent translation is highly efficient. What might be the advantage of having internal ribosome binding sites if the cap permits the initiation of translation and all eukaryotic mRNAs are capped?

8. Some research suggests that translation of proteins is regulated by ribosome stalling: ribosomes attach to mRNA and then stall; elongation commences only in response to a specific stimulus. What might be an advantage to gene regulation by ribosome stalling?

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Summarize the role of gene regulation in producing differences between humans and chimpanzees by creating a concept map that includes the following terms: humans, chimpanzees, phenotypic differences, genetic differences, regulatory sequences, genes, transcription factors, KRAB-ZFPs, rapid evolution, natural selection, regulatory networks.

Section 17.1

2. Outline the effects of changes in chromatin structure on gene expression by drawing a concept map that includes at least the following concepts: chromatin structure, DNase I hypersensitive sites, chromatin-remodeling complexes, methylation of histones, acetylation of histones, methyltransferases, demethylases, reposition of nucleosomes, chromatin immunoprecipitation, DNA methylation, acetyltransferase

enzymes, deacetylases, CpG nucleotides, histone code, increase transcription, decrease transcription.

Section 17.3

3. Create a concept map that summarizes the different types of regulatory elements that control initiation and elongation of transcription in eukaryotes.

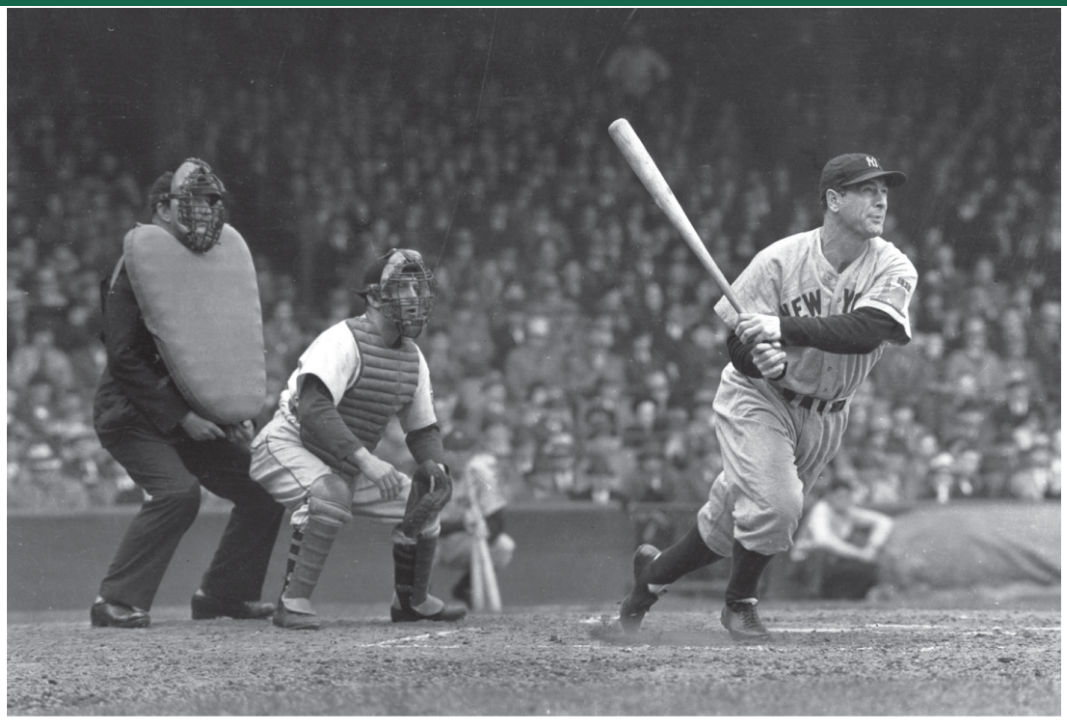
Section 17.5

4. How do small RNAs regulate expression of genes in eukaryotes? Draw a concept map that outlines the different mechanisms of gene regulation by RNA interference.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 18

Gene Mutations and DNA Repair



Transcendental Graphics/Getty Images.

Lou Gehrig at bat. Gehrig, who played baseball for the New York Yankees from 1923 to 1939, was diagnosed with amyotrophic lateral sclerosis, a disease that in some people is caused by an expanding nucleotide repeat mutation.

Lou Gehrig and Expanding Nucleotide Repeats

Lou Gehrig was the finest first baseman ever to play major league baseball. A left-handed power hitter who grew up in New York City, Gehrig played for the New York Yankees from 1923 to 1939. Throughout his career, he lived in the shadow of his teammates Babe Ruth and Joe DiMaggio, but Gehrig was a great hitter in his own right: he compiled a lifetime batting average of .340 and drove in more than 100 runs every season for 13 years. During his career, he batted in 1991 runs and hit a total of 23 grand slams (home runs with bases loaded). But Gehrig's greatest baseball record, which stood for more than 50 years and has been broken only once—by Cal Ripken, Jr., in 1995—is his record of playing 2130 consecutive games.

In the 1938 baseball season, Gehrig fell into a strange slump. For the first time since his rookie year, his batting average dropped below .300, and in the World Series that year, he managed only four hits—all singles. Nevertheless, he finished the season convinced that he was undergoing a temporary slump that he would overcome in the next season. He returned to training camp in 1939 with high spirits. When the season began, however, it was clear to everyone that something was terribly wrong. Gehrig had no power in his swing; he was awkward and clumsy at first base. His condition worsened, and on May 2, he voluntarily removed himself from the lineup. The Yankees sent Gehrig to the Mayo Clinic for diagnosis. On June 20, his medical report was made public: Lou Gehrig was suffering from a rare, progressive disease known as amyotrophic

lateral sclerosis (ALS). Within two years, he was dead. Since then, ALS has commonly been known as Lou Gehrig disease.

Gehrig experienced symptoms typical of ALS: progressive weakness and wasting of skeletal muscles due to degeneration of the motor neurons. Most cases of ALS are sporadic, appearing in people with no family history of the disease. However, about 10% of cases run in families, and in these cases the disease is inherited as an autosomal dominant trait. ALS shares a number of features in common with another neurological disease called frontotemporal dementia (FTD); in fact, FTD occurs alongside ALS in some families, suggesting a common genetic basis underlying these two disorders.

Mutations in several genes can cause familial cases of ALS and FTD, the most common of which occur in a gene on chromosome 9 called *chromosome 9 open reading frame 72* (*C9orf72*). The alterations of *C9orf72* that are associated with ALS and FTD belong to an unusual group of mutations called expanding nucleotide repeats, in which the number of copies of a set of nucleotides is increased. Most people have somewhere between 2 and 23 repeats of the nucleotide sequence GGGGCC in their *C9orf72* gene, but this number is massively expanded in some people with ALS, who typically possess 700 to 1600 repeats of the sequence.

How the expansion of the GGGGCC repeat in *C9orf72* leads to symptoms of ALS and FTD is unknown, but recent research

demonstrates that the repeats are translated into one or more proteins that are toxic to nerve cells. The repeats are translated in an unusual and intriguing way: the GGGGCC sequences on both the template and the nontemplate strands of the gene are transcribed into RNAs that are translated without a start codon. Because there is no start codon to set the reading frame, all three reading frames on both mRNAs are translated into proteins, resulting in five proteins, each with a different series of repeating dipeptides: glycine-alanine, glycine-proline, proline-alanine, glycine-arginine, and proline-arginine.

To determine how the repeats might produce the disease, geneticists engineered a series of premature stop codons into the template and nontemplate strands of the *C9orf72* gene so that RNA would be transcribed from the repeats but, because of the engineered stop codons, would not be translated into a protein. They inserted both the original repeat sequence and the engineered repeat sequence into fruit flies. The unaltered repeats caused neurodegeneration and early death in the fruit flies, but the engineered repeats had no effect. These results suggest that the toxicity of the repeat sequence was the result of the protein it encoded and not simply a product of the RNA alone (although the RNA may also be somewhat toxic). Further research suggested that proteins with the glycine-arginine and proline-arginine dipeptides are responsible for the neurodegeneration that occurs in ALS and FTD. The toxicity of these proteins may result from the fact that

they mimic RNA-binding proteins and interfere with splicing of pre-mRNA and the processing of rRNA. This research provides important insight into the pathology of these diseases and suggests possible future targets for treatment.

THINK-PAIR-SHARE

- Propose some ways that the new information provided by research on the role of the GGGGCC repeat in ALS might be used to design potential treatments for the disease.
- Using the genetic code illustrated in Figure 15.10, show how translation of the GGGGCC repeat without a start codon results in the production of five proteins with different dipeptide repeats. (Hint: Consider all reading frames of the two RNAs copied from this sequence.)

The story of ALS and expanding nucleotide repeats illustrates the central importance of studying mutations: the analysis of mutants is often a source of key insights into diseases and important biological processes. This chapter focuses on gene mutations—on how these errors in genetic instructions arise and how they are studied. We begin with a brief examination of the different types of mutations, including their phenotypic effects, how they can be suppressed, and their rates of occurrence. The next section explores how mutations can arise spontaneously during and after the course of DNA replication, as well as how chemicals and radiation can induce them. After discussing the analysis of mutations, we turn to transposable elements: DNA sequences that are capable of moving within the genome and that often produce

mutations when they do so. Finally, we take a look at DNA repair and some of the diseases that arise when DNA repair is defective.

18.1 Mutations Are Inherited Alterations in the DNA Sequence

DNA is a highly stable molecule that is replicated with amazing accuracy (as we saw in Chapters 10 and 12), but changes in DNA structure and errors of replication do take place. A **mutation** is defined as an inherited change in the DNA sequence of genetic information; the descendants that inherit the change may be cells or organisms.

The Importance of Mutations

Mutations are both the sustainer of life and the cause of great suffering. On the one hand, mutation is the source of all genetic variation, the raw material of evolution. The ability of organisms to adapt to environmental change depends on the presence of genetic variation in natural populations, and genetic variation is produced by mutation. On the other hand, many mutations have detrimental effects, and mutation is the source of many diseases and disorders.

Much of the study of genetics focuses on how genetic variants produced by mutation are inherited; genetic crosses are meaningless if all individual members of a species are identically homozygous for the same alleles. Much of Gregor Mendel's success in unraveling the principles of inheritance can be traced to his use of carefully

selected variants of the garden pea (see Chapter 3). Similarly, Thomas Hunt Morgan and his students discovered many basic principles of genetics by analyzing mutant fruit flies (discussed in Chapter 4).

Mutations are also useful for examining fundamental biological processes. Finding or creating mutations that affect different components of a biological system and studying their effects can often lead to a better understanding of the system. This method, referred to as genetic dissection, is analogous to figuring out how an automobile works by breaking different parts of a car and observing the effects; for example, smash the radiator and the engine overheats, revealing that the radiator cools the engine. The use of mutations to disrupt function can likewise be a source of insight into biological processes. For example, geneticists have begun to unravel the molecular details of development by studying mutations that interrupt various embryonic stages in *Drosophila* (see Chapter 22). Scientists have also used analysis of mutations to reveal the different parts of the *lac* operon (discussed in Chapter 16) and how they function in gene regulation. Although breaking "parts" to determine their function might seem like a crude approach to understanding a system, it is actually a very powerful one and has been used extensively in biochemistry, developmental biology, physiology, and behavioral science. But this method is *not* recommended for learning how your car works!

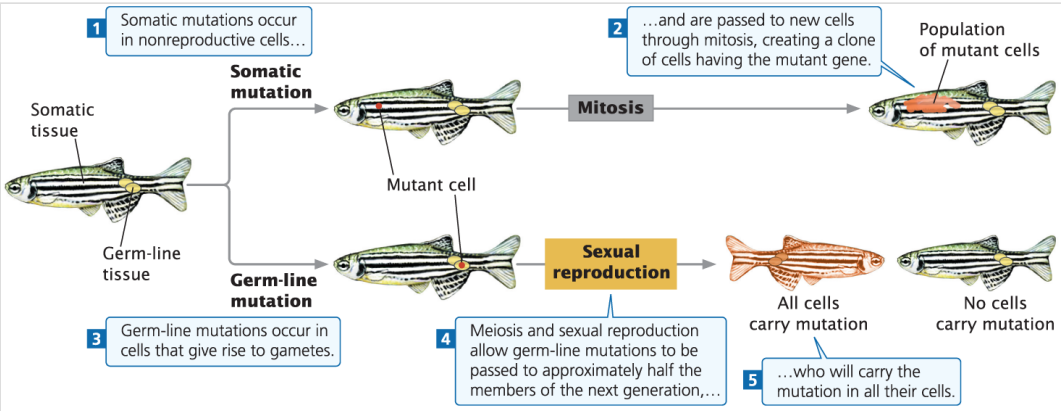
THINK-PAIR-SHARE Question 1

CONCEPTS

Mutations are heritable changes in DNA. They are essential to the study of genetics and are useful in many other biological fields.

Categories of Mutations

In multicellular organisms, we can distinguish between two broad categories of mutations: somatic mutations and germ-line mutations. **Somatic mutations** arise in somatic tissues, which do not produce gametes (**Figure 18.1**). When a somatic cell with a mutation divides (by mitosis), the mutation is passed on to the daughter cells, leading to a population of genetically identical cells (a clone). The earlier in development that a somatic mutation takes place, the larger the clone of cells that contain the mutation will be.



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18.1 The two basic classes of mutations are somatic mutations and germ-line mutations.

Description
The illustration shows a green fish on the left. The center of the fish is labeled somatic tissue and the area at the end of the fins is labeled germ line tissue. An arrow from the initial fish bifurcates. The top arrow shows somatic mutation. A mutant cell is present in a fish in its somatic tissue. Mitosis occurs and a green fish obtained shows a large population of mutant cells in its somatic tissue. The bottom arrow from the initial fish shows germ-line mutation. A mutant cell is present in a fish in its germ line tissue. Sexual reproduction occurs and two fish obtained on the right. A red-fish's cells all carry the mutation and a green fish has no cells that carry mutation. Captions throughout read, Somatic mutations occur in non reproductive cells and are passed to new cells through mitosis, creating a clone of cells having the mutant gene. Germ-line mutations occur in cells that give rise to gametes. Meiosis and sexual reproduction allow germ-line mutations to be passed to approximately half the members of the next generation who will carry the mutation in all their cells.

Because of the huge number of cells present in a typical eukaryotic organism, somatic mutations are numerous. For example, there are about 10^{14} cells in the human body. Typically, a mutation arises once in every million cell divisions, so hundreds of millions of somatic mutations must arise in each person. Many somatic mutations have no obvious effect on the phenotype of the organism because the function of the mutant cell is taken over by a normal cell, or the mutant cell dies and is replaced by normal cells. However, cells with a somatic mutation that stimulates cell division can increase in number and spread; this type of mutation can give rise to cells with a selective advantage and is the basis for cancer (see Chapter 23). Somatic mutations are also associated with some other diseases,

including hemimegalencephaly, in which just one hemisphere of the brain is enlarged, usually resulting in epilepsy. And somatic mutations can lead to mosaicism, in which different tissues within the body have different genetic information (see Chapter 6).

It has long been assumed that somatic mutations accumulate with age, giving rise to cancer and aging, but measuring the frequency of somatic mutations has been difficult. In recent years, the development of methods to accurately sequence whole genomes of single cells (see Chapter 20) and to correct for sequencing artifacts has made it possible to reliably estimate numbers of somatic mutations. The sequencing of individual neurons from the brains of people of different ages has demonstrated that the number of somatic mutations increases with age, and individuals with genetic diseases that interfere with DNA repair (see Section 18.5) have higher rates of somatic mutations than expected for their age.

Germ-line mutations arise in cells that ultimately produce gametes. A germ-line mutation can be passed to future generations, producing offspring that carry the mutation in all their somatic and germ-line cells (see Figure 18.1). When we speak of mutations in multicellular organisms, we're usually talking about germ-line mutations.

Historically, mutations have been partitioned into those that affect a single gene, called *gene mutations*, and those that affect the number or structure of chromosomes, called *chromosome mutations*. This

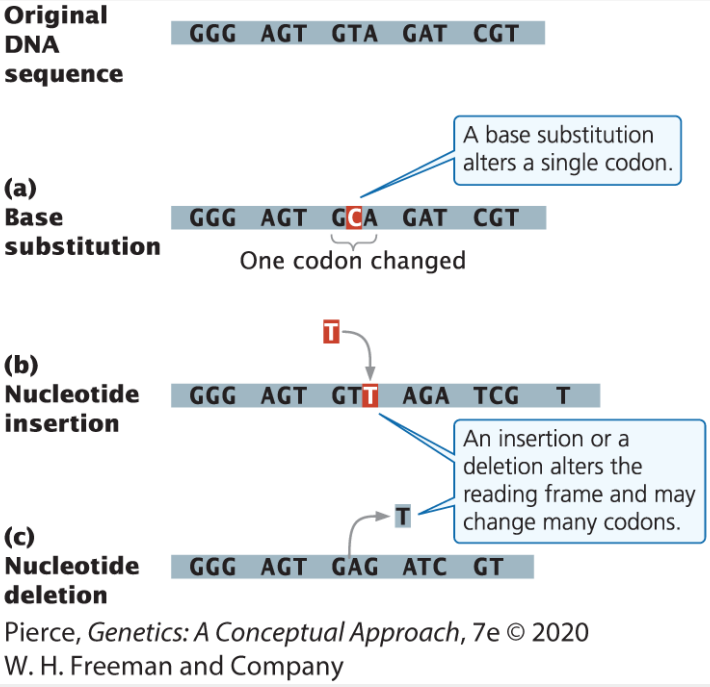
distinction arose because chromosome mutations could be observed directly, by looking at chromosomes with a microscope, whereas gene mutations could be detected only by observing their phenotypic effects. Now DNA sequencing allows direct observation of gene mutations, and chromosome mutations are distinguished from gene mutations somewhat arbitrarily on the basis of the size of the DNA lesion. Nevertheless, it is practical to use *chromosome mutation* for a large-scale genetic alteration that affects chromosome structure or the number of chromosomes and to use **gene mutation** for a relatively small DNA lesion that affects a single gene. This chapter focuses on gene mutations; chromosome mutations were discussed in Chapter 8.

Types of Gene Mutations

There are a number of ways to classify gene mutations. Some classification schemes are based on the nature of the phenotypic effect, others are based on the causative agent of the mutation, and still others focus on the molecular nature of the defect. Here we will categorize mutations primarily on the basis of their molecular nature, but we will also encounter some terms that relate the causes and the phenotypic effects of mutations.

BASE SUBSTITUTIONS

The simplest type of gene mutation is a **base substitution**, the alteration of a single nucleotide in the DNA (**Figure 18.2a**). There are two types of base substitutions. In a **transition**, a purine is replaced by a different purine or, alternatively, a pyrimidine is replaced by a different pyrimidine (**Figure 18.3**). In a **transversion**, a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine. The number of possible transversions (see Figure 18.3) is twice the number of possible transitions, but transitions arise more frequently because transforming a purine into a different purine or a pyrimidine into a different pyrimidine is easier than transforming a purine into a pyrimidine, or vice versa. **› TRY PROBLEM 19**



18.2 Three basic types of gene mutations are base substitutions, insertions, and deletions.

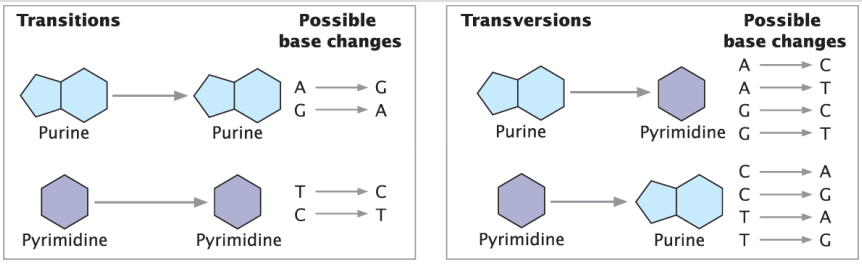
Description

The illustration shows an original D N A sequence at the top. The sequence is as follows: G G G, A G T, G T A, G A T, C G T.

Part a below shows base substitution. The new sequence is as follows: G G G, A G T, G C (highlighted with a note that reads, one codon changed) A, G A T, C G T. A caption reads, A base substitution alters a single codon.

Part b below shows Nucleotide insertion. The new sequence is as follows: G G G, A G T, G T T (highlighted), A G A, T C G, T. Nucleotide T is inserted.

Part c below shows Nucleotide deletion. The new sequence is as follows: G G G, A G T, G A (nucleotide T is deleted) G, A T C, G T. A caption corresponding to the highlighted T in part b and the T being removed from G A G reads, An insertion or a deletion alters the reading frame and may change many codons.



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18.3 A transition is the substitution of a purine for a purine or of a pyrimidine for a pyrimidine; a transversion is the substitution of a pyrimidine for a purine or of a purine for a pyrimidine.

Description

The first part shows two types of transitions. The first transition shows a purine, a five membered ring fused with a six membered ring, converting to another purine. The possible base changes include: A to G and G to A. The second transition below shows a pyrimidine, a six membered ring, converts to another pyrimidine. The possible base changes include: T to C and C to T.

The second part shows two types of transversions. The first transversion shows a purine converting to a pyrimidine. The possible base changes include: A to C, A to T, G to C, and G to T. The second transversion below shows a pyrimidine converting to a purine.

Pyrimidine is a six membered ring whereas Purine is a five membered ring fused with a six membered ring. The possible base changes include: C to A, C to G, T to A, and T to G.

INSERTIONS AND DELETIONS

Another class of gene mutations consists of **insertions** and **deletions** (collectively called *indels*): the addition or removal, respectively, of one or more nucleotide pairs (**Figure 18.2b and c**). Although base substitutions are often assumed to be the most common type of mutation, molecular analysis has revealed that insertions and deletions are often more frequent. Insertions and deletions within sequences that encode proteins may lead to **frameshift mutations**: changes in the reading frame (see pp. 445–446 in Chapter 15) of the gene. Frameshift mutations usually alter all amino acids encoded by the nucleotides following the mutation, so they generally have drastic effects on the phenotype. Some frameshifts also introduce premature stop codons, terminating protein synthesis early and resulting in a shortened (truncated) protein. Not all insertions and deletions lead to frameshifts, however; insertions and deletions consisting of any multiple of three nucleotides leave the reading frame intact, although the addition or removal of one or more amino acids may still affect the phenotype. Indels that do not affect the reading frame are called **in-frame insertions** and **in-frame deletions**.

CONCEPTS

Gene mutations are changes in a single gene. They can be base substitutions (in which a single pair of nucleotides is altered) or insertions or deletions (in which nucleotides are added or removed). A base substitution can be a transition (substitution of like bases) or a transversion (substitution of unlike bases). Insertions and deletions often lead to a change in the reading frame of a gene.

✔ CONCEPT CHECK 1

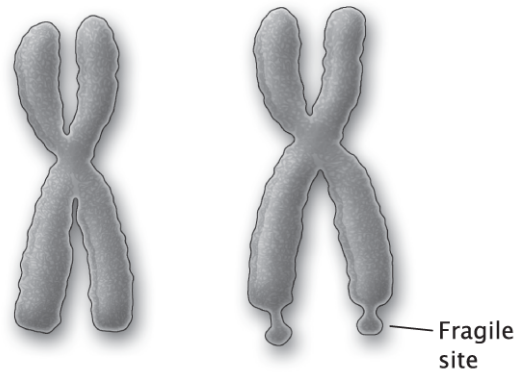
Which of the following changes is a transition base substitution?

a. Adenine is replaced by thymine.
b. Cytosine is replaced by adenine.
c. Guanine is replaced by adenine.
d. Three nucleotide pairs are inserted into DNA.

EXPANDING NUCLEOTIDE REPEATS

Mutations in which the number of copies of a set of nucleotides increases are called **expanding nucleotide repeats**. As discussed in the introduction to the chapter, this is the type of mutation that is responsible for some familial cases of ALS. Expanding nucleotide repeats were first discovered in 1991 in a gene called *FMR-1*, which causes fragile-X syndrome, the most common hereditary cause of intellectual disability. The disorder is so named because when specially treated cells from people with the condition are examined under a microscope, the tip of each long arm of the X chromosome is attached by a slender-appearing part of the chromosome (**Figure 18.4**). The normal *FMR-1* allele (not containing the mutation) has 54 or fewer copies of the sequence CGG, but in people with fragile-X

syndrome, the allele may harbor hundreds or even thousands of copies.



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18.4 Fragile-X syndrome is associated with a characteristic constriction (fragile site) on the long arm of the X chromosome.

Description
The illustration shows an X shaped chromosome with almost equal arms. The illustration shows another X shaped chromosome with almost equal arms. Both the long arms of this chromosome have a tiny extension below them with a round lump at the end labeled, fragile site.

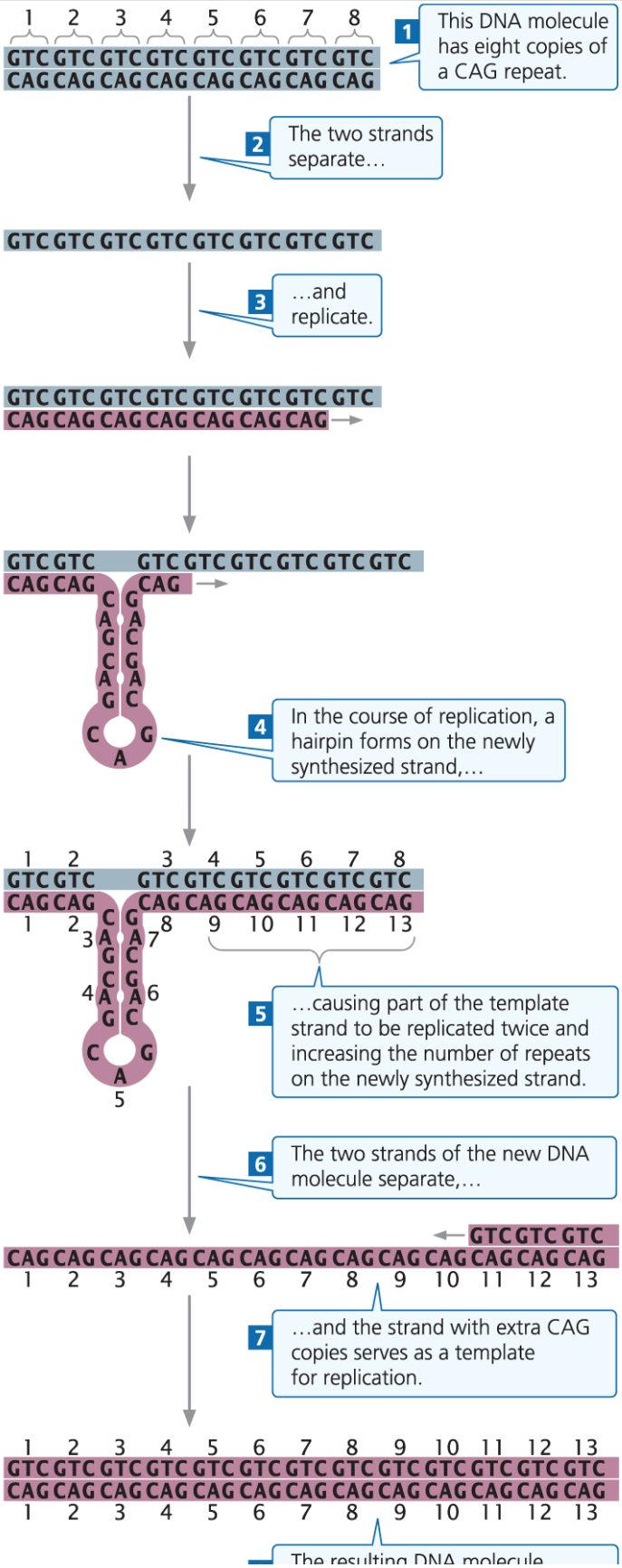
Expanding nucleotide repeats have been found in almost 30 human diseases, several of which are listed in Table 18.1. Most of these diseases are caused by the expansion of a set of three nucleotides (called a trinucleotide), most often CNG, where N can be any nucleotide. However, some diseases are caused by repeats of four, five, and even twelve nucleotides. The number of copies of the repeat often correlates with the severity or age of onset of the disease. The number of copies of the repeat also correlates with its

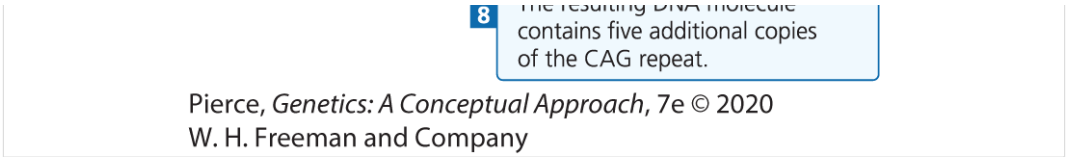
instability: when more repeats are present, the probability of expansion to even more repeats increases. This association between the number of copies of nucleotide repeats, the severity of the resulting disease, and the probability of expansion leads to a phenomenon known as anticipation (see Chapter 5), in which diseases caused by expanding nucleotide repeats become more severe in each generation. Less commonly, the number of nucleotide repeats may decrease within a family. Expanding nucleotide repeats have also been observed in some microbes and plants.

TABLE 18.1 Examples of human genetic diseases caused by expanding nucleotide repeats

		Number of Copies of Repeat	
Disease	**Repeated Sequence**	**Normal Range**	**Disease Range**
Spinal and bulbar muscular atrophy	CAG	11–33	40–62
Fragile-X syndrome	CGG	6–54	50–1500
Jacobsen syndrome	CGG	11	100–1000
Spinocerebellar ataxia (several types)	CAG	4–44	21–130
Autosomal dominant cerebellar ataxia	CAG	7–19	37–220
Myotonic dystrophy	CTG	5–37	44–3000
Huntington disease	CAG	9–37	37–121
Friedreich ataxia	GAA	6–29	200–900
Dentatorubral-pallidoluysian atrophy	CAG	7–25	49–75
Myoclonus epilepsy of the Unverricht–Lundborg type	CCCCGCCCCGCG	2–3	12–13

Increases in the number of nucleotide repeats can produce disease symptoms in different ways. In several diseases (e.g., Huntington disease), the nucleotide expansion occurs within the coding part of a gene, producing a toxic protein that has extra glutamine (the amino acid encoded by CAG). In other diseases, the repeat is outside the coding region of a gene and affects its expression. For example, in fragile-X syndrome, the additional copies of the nucleotide repeat cause the DNA to become methylated, which turns off the transcription of an essential gene. In other cases, it appears that RNA transcribed from the repeats may itself be toxic or may be abnormally translated into short proteins that cause disease symptoms (see introduction to this chapter). Expansion of nucleotide repeats occurs in the course of DNA replication and appears to be related to the formation of hairpins and other special secondary structures that form in single-stranded DNA consisting of nucleotide repeats. Such structures may interfere with normal replication by causing strand slippage, misalignment of the sequences, or stalling of replication. One model of how hairpin formation in the repeats might result in their expansion is shown in Figure 18.5. Watching (A) **Animation 18.1** will help you understand how copies of nucleotide repeats increase in number. Other models of repeat expansion that occur through transcription and DNA repair have also been proposed. Many aspects of this phenomenon are not well understood, including why repeat expansion occurs in some people and not in others.


1 2 3 4 5 6 7 8
GTC GTC GTC GTC GTC GTC GTC GTC
CAG CAG CAG CAG CAG CAG CAG CAG
1 This DNA molecule has eight copies of a CAG repeat.
2 The two strands separate…
GTC GTC GTC GTC GTC GTC GTC GTC
3 …and replicate.
GTC GTC GTC GTC GTC GTC GTC GTC
CAG CAG CAG CAG CAG CAG CAG
GTC GTC GTC GTC GTC GTC GTC GTC
CAG CAG CAG
4 In the course of replication, a hairpin forms on the newly synthesized strand,…
1 2 3 4 5 6 7 8
GTC GTC GTC GTC GTC GTC GTC GTC
CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG
1 2 3 4 5 6 7 8 9 10 11 12 13
5 …causing part of the template strand to be replicated twice and increasing the number of repeats on the newly synthesized strand.
6 The two strands of the new DNA molecule separate,…
GTC GTC GTC
CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG
1 2 3 4 5 6 7 8 9 10 11 12 13
7 …and the strand with extra CAG copies serves as a template for replication.
1 2 3 4 5 6 7 8 9 10 11 12 13
GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC
CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG
1 2 3 4 5 6 7 8 9 10 11 12 13
The resulting DNA molecule



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18.5 A model of how the number of copies of a nucleotide repeat may increase in replication.

Description

The illustration shows a D N A double strand at the top. The top strand shows the following sequence: G T C repeated eight times and labeled 1 through 8. The bottom strand with complementary bases is as follows: C A G repeated eight times. A caption reads, This D N A molecule has eight copies of a C A G repeat. The two strands separate and replicate. The bottom strand separates from the top strand. The top strand remains below. Replication occurs. The double strand obtained below has same top strand as in the beginning. The sequence of newly synthesized bottom strand is as follows: C A G repeated seven times. An arrow to the right side is present after this sequence. The bottom strand forms a hairpin loop below, where 5 C A G repeats are involved. As the 5 G T C repeats of the top strand do not have complementary bases, the bottom strand with hairpin loop gets elongated. The bottom strand now has 5 addition C A G repeats, labeled 9 through 13, corresponding to G T C repeats in first strand labeled 4 through 8. A caption reads, In the course of replication, a hairpin forms on the newly synthesized strand causing part of the template strand to be replicated twice and increasing the number of repeats on the newly synthesized strand. Both the strands separate, only the bottom strand remains below. A caption reads, The two strands of the new D N A molecule separate, and the strand with extra C A G repeats serves as a template for replication. Now the remaining bottom strand with C A G repeats, labeled 1 through 13, serves as a template and a complementary strand is synthesized above. The top strand is synthesized from right to left and has G T C repeats. The newly synthesized top strand elongates and now has G T C repeats, labeled 1 through 13 for the C A G repeats for the template strand below. A caption reads, The resulting D N A molecule contains five additional copies of the C A G repeat.

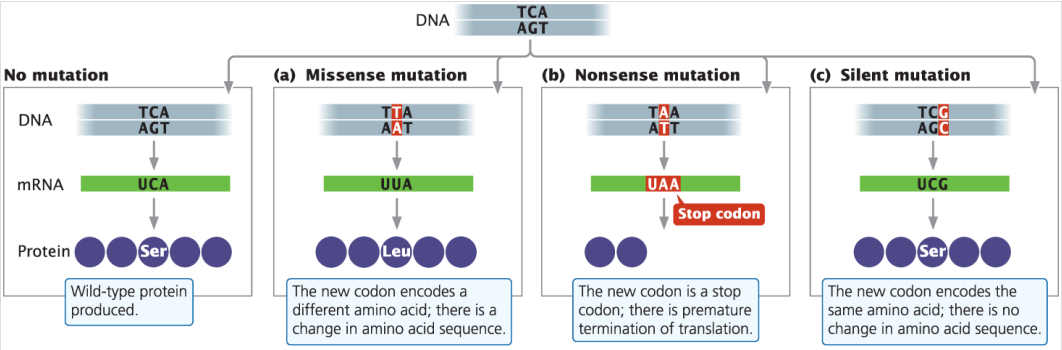
CONCEPTS

Expanding nucleotide repeats are mutations in which the number of copies of a set of repeated nucleotides increases over time. These mutations are associated with several human genetic diseases.

Functional Effects of Mutations

Another way that mutations are classified is by their functional effects. At the most general level, we can distinguish a mutation on the basis of its phenotype compared with the wild-type phenotype. A mutation that alters the wild-type phenotype is called a **forward mutation**, whereas a **reverse mutation** (a *reversion*) changes a mutant phenotype back into the wild type.

Geneticists use other terms to describe the effects of mutations on protein structure. A base substitution that results in a different amino acid in the protein is referred to as a **missense mutation** (**Figure 18.6a**). A **nonsense mutation** changes a sense codon (one that specifies an amino acid) into a nonsense codon (one that terminates translation), as shown in **Figure 18.6b**. If a nonsense mutation occurs early in the mRNA sequence, the protein will be truncated and usually nonfunctional.



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18.6 Base substitutions can cause (a) missense, (b) nonsense, or (c) silent mutations.

Description

The top center of the illustration shows a D N A double strand. The top strand has the following sequence: T, C, A. The bottom strand has the following sequence: A, G, T. A downward arrow from the double strand divides into four arrows below.

The first arrow, at bottom left, shows no mutation. A double D N A strand is present at the top. The sequence of the top strand is as follows: T, C, A. The sequence of the bottom strand is as follows: A, G, T. An m R N A is produced below with the following sequence: U, C, A. A protein is formed below with serine (Ser), at the center of the chain. A caption below reads, Wild-type protein produced.

The second arrow on the right shows (a) Missense mutation. A double D N A strand is present at the top. The sequence of the top strand is as follows: T, T (highlighted), A. The sequence of the bottom strand is as follows: A, A (highlighted), T. An m R N A is produced below with the following sequence: U, U, A. A protein is formed below with leucine (Leu), at the center of the chain. A caption below reads, The new codon encodes a different amino acid; there is a change in amino acid sequence.

The third arrow on the right shows (b) Non sense mutation. A double D N A strand is present at the top. The sequence of the top strand is as follows: T, A (highlighted), A. The sequence of the bottom strand is as follows: A, T (highlighted), T. An m R N A is produced below with the following sequence: U (highlighted), A (highlighted), A (highlighted). The m R N A is labeled, stop codon. A protein chain is formed below. A caption below reads, The new codon is a stop codon; there is premature termination of translation.

The fourth arrow on the right shows (c) Silent mutation. A double D N A strand is present at the top. The sequence of the top strand is as follows: T, C, G (highlighted). The sequence of the bottom strand is as follows: A, G, C (highlighted). An m R N A is produced below with the following sequence: U, C, G. A protein is formed below with serine (Ser), at the center of the chain. A caption below reads, The new codon encodes the same amino acid; there is no change in amino acid sequence.

Because of the redundancy of the genetic code, some different codons specify the same amino acid. A **silent mutation** changes a codon to a synonymous codon that specifies the same amino acid (**Figure 18.6c**), altering the DNA sequence without changing the amino acid sequence of the protein. Not all silent mutations, however, are truly silent: some do have phenotypic effects. They may have phenotypic effects, for example, when different tRNAs (called isoaccepting tRNAs; see Chapter 15) bind to different synonymous codons. Because some isoaccepting tRNAs are more abundant than others, which synonymous codon is used may affect the rate of protein synthesis. The rate of protein synthesis, in turn, can influence the phenotype by affecting the amount of protein present in the cell and, in a few cases, the folding of the protein. Some silent mutations alter nucleotides that serve as binding sites for regulatory proteins or alter sequences near exon–intron junctions that affect mRNA splicing (see Chapter 14). Still other silent mutations can influence the binding of miRNAs to complementary sequences in the mRNA, which determines whether the mRNA is translated (see Chapter 14).

A **neutral mutation** is a missense mutation that alters the amino acid sequence of a protein but does not significantly change its function. Neutral mutations occur when one amino acid is replaced by another that is chemically similar, or when the affected amino acid has little influence on protein function. For example, some neutral mutations occur in the genes that encode hemoglobin; although these mutations alter the amino acid sequence of hemoglobin, they do not affect its ability to transport oxygen.

Loss-of-function mutations cause the complete or partial absence of normal protein function. A loss-of-function mutation is one that so alters the structure of the protein that the protein no longer works correctly, or one that occurs in regulatory regions that affect the transcription, translation, or splicing of the protein. Loss-of-function mutations are frequently recessive, in which case a diploid individual must be homozygous for the mutation before the effects of the loss of the functional protein can be exhibited. The mutations that cause cystic fibrosis are loss-of-function mutations: these mutations produce a nonfunctional form of the CFTR protein, which normally regulates the movement of chloride ions into and out of the cell (see Chapter 5).

In contrast, a **gain-of-function mutation** causes the cell to produce a protein or gene product whose function is not normally present. The result could be an entirely new gene product or one produced in an inappropriate tissue or at an inappropriate time in development. For example, a mutation in a gene that encodes a receptor for a growth

factor might cause the mutated receptor to stimulate growth all the time, even in the absence of the growth factor. Gain-of-function mutations are frequently dominant in their expression because a single copy of the mutation leads to the presence of a new gene product.

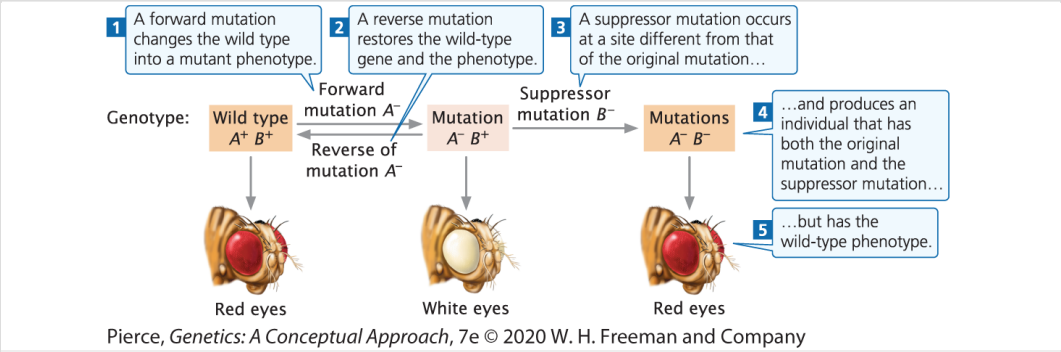
Other mutations are **conditional mutations**, which are expressed only under certain conditions. For example, some conditional mutations affect the phenotype only at elevated temperatures. Still others are **lethal mutations**, which cause premature death (see Chapter 5). ❯ **TRY PROBLEM 23**

THINK-PAIR-SHARE Question 2

Suppressor Mutations

A **suppressor mutation** is a genetic change that hides or suppresses the effect of another mutation. This type of mutation is different from a reverse mutation, where the mutated site is changed back to the original wild-type sequence (**Figure 18.7**). A suppressor mutation occurs at a site distinct from the site of the original mutation; thus, an individual with a suppressor mutation is a double mutant, possessing both the original mutation and the suppressor mutation but exhibiting the phenotype of the nonmutated wild type.

Geneticists distinguish between two classes of suppressor mutations: intragenic and intergenic.



18.7 Relation of forward, reverse, and suppressor mutations.

Description

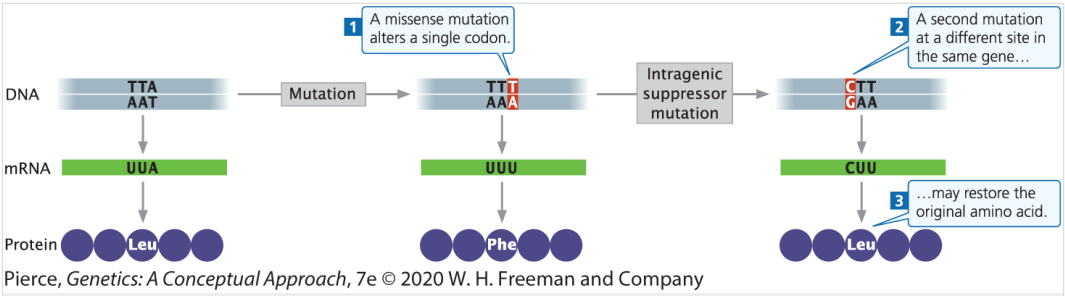
The illustration shows a wild type genotype on the left. The wild type genotype is as follows: big A superscript positive big B superscript positive. A downward arrow shows a fly with red, large eyes. A forward arrow from the wild type genotype big A superscript positive big B superscript positive shows a forward mutation big A superscript negative and results in mutation big A superscript negative big B superscript positive. A caption reads, A forward mutation changes the wild type into a mutant phenotype.

A backward arrow from the mutation big A superscript negative big B superscript positive shows reverse of mutation big A superscript negative and leads to wild type genotype big A superscript positive big B superscript positive. A caption reads, A reverse mutation restores the wild-type gene and the phenotype. A downward arrow from the mutation leads to a fly with white, large eyes.

A forward arrow from the mutation big A superscript negative big B superscript positive shows suppressor mutation big B superscript negative and leads to mutations big A superscript negative big B superscript negative. A downward arrow from the mutations big A superscript negative big B superscript negative leads to a fly with red, large eyes. A caption reads, A suppressor mutation occurs at a site different from that of the original mutation and produces an individual that has both the original mutation and the suppressor mutation but has the wild-type phenotype.

INTRAGENIC SUPPRESSOR MUTATIONS

An **intragenic suppressor mutation** takes place in the same gene that contains the mutation being suppressed. It may work in any of several ways. The suppressor may change a second nucleotide in the same codon altered by the original mutation, producing a codon that specifies the same amino acid that was specified by the original, nonmutated codon (Figure 18.8).



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18.8 An intragenic suppressor mutation occurs in the gene containing the mutation being suppressed.

Description

The illustration starts with a D N A double strand at the left.

The sequence of the top strand is as follows: T, T, A. The sequence of the bottom strand is as follows: A, A, T. An m R N A is produced below with the following sequence: U, U, A. A protein is formed below with leucine (Leu), at the center of the chain. A forward arrow from the D N A double strand represents mutation and points to another D N A double strand, at the bottom center.

The sequence of the top strand is as follows: T, T, T (highlighted). The sequence of the bottom strand is as follows: A, A, A (highlighted). A caption reads, A missense mutation alters a single codon. An m R N A is produced below with the following sequence: U, U, U. A protein is formed below with Phenylalanine (Phe), at the center of the chain. A forward

arrow from the D N A double strand with missense mutation represents Intragenic suppressor mutation and points to another D N A double strand, at the bottom right.

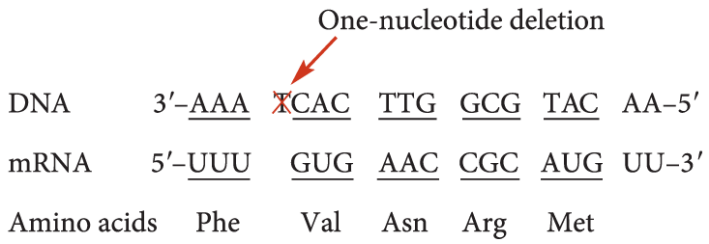
The sequence of the top strand is as follows: C (highlighted), T, T. The sequence of the bottom strand is as follows: G (highlighted), A, A. An m R N A is produced below with the following sequence: C, U, U. A protein is formed below with Leucine (Leu), at the center of the chain. A caption reads, A second mutation at a different site in the same gene may restore the original amino acid.

Intragenic suppressors may also work by suppressing a frameshift mutation. If the original mutation, for example, is a one-base deletion, then the addition of a single base elsewhere in the gene will restore the former reading frame. Consider the following nucleotide sequence on the template strand of DNA and the amino acids that it encodes:

DNA	3′–AAA	TCA	CTT	GGC	GTA	CAA–5′
mRNA	5′–UUU	AGU	GAA	CCG	CAU	GUU–3′
Amino acids	Phe	Ser	Glu	Pro	His	Val

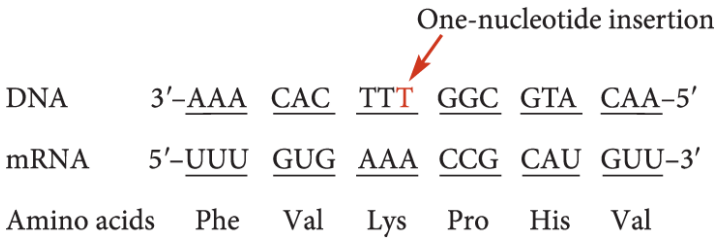
Description
The D N A strand shows the following sequence: 3 prime-A A A, T C A, C T T, G G C, G T A, C A A-5 prime. The m R N A obtained below shows the following sequence: 5 prime-U U U, A G U, G A A, C C G, C A U, G U U-3 prime. The corresponding amino acids shown below are as follows: Phe, Ser, Glu, Pro, His, Val. The nucleotides are underlined in groups of three nucleotides.

Suppose that a one-base deletion occurs in the first nucleotide of the second codon. This deletion shifts the reading frame by one nucleotide and alters all the amino acids that follow the mutation:



Description
The D N A strand at top right shows the following sequence: 3 prime-A A A, T (crossed out) C A C, T T G, G C G, T A C, A A 5-prime. A caption from crossed out nucleotide reads, one-nucleotide deletion. the m R N A obtained below shows the following sequence: 5 prime-U U U, G U G, A A C, C G C, A U G, U U-3 prime. The corresponding amino acids shown below are as follows: Phe, Val, Asn, Arg, Met. The nucleotides are underlined in groups of three nucleotides.

If a single nucleotide is added to the third codon (the suppressor mutation), the reading frame is restored, although two of the amino acids differ from those specified by the original sequence:



Description
The D N A strand at top right shows the following sequence: 3 prime-A A A, C A C, T T T (highlighted), G G C, G T A, C A A 5-prime. The highlighted nucleotide shows a caption that reads, one nucleotide insertion. The m R N A obtained below shows the following sequence: 5 prime-U U U, G U G, A A A, C C G, C A U, G U U-3 prime. The corresponding amino acids shown below are as follows: Phe, Val, Lys, Pro, His, Val. The nucleotides are underlined in groups of three nucleotides.

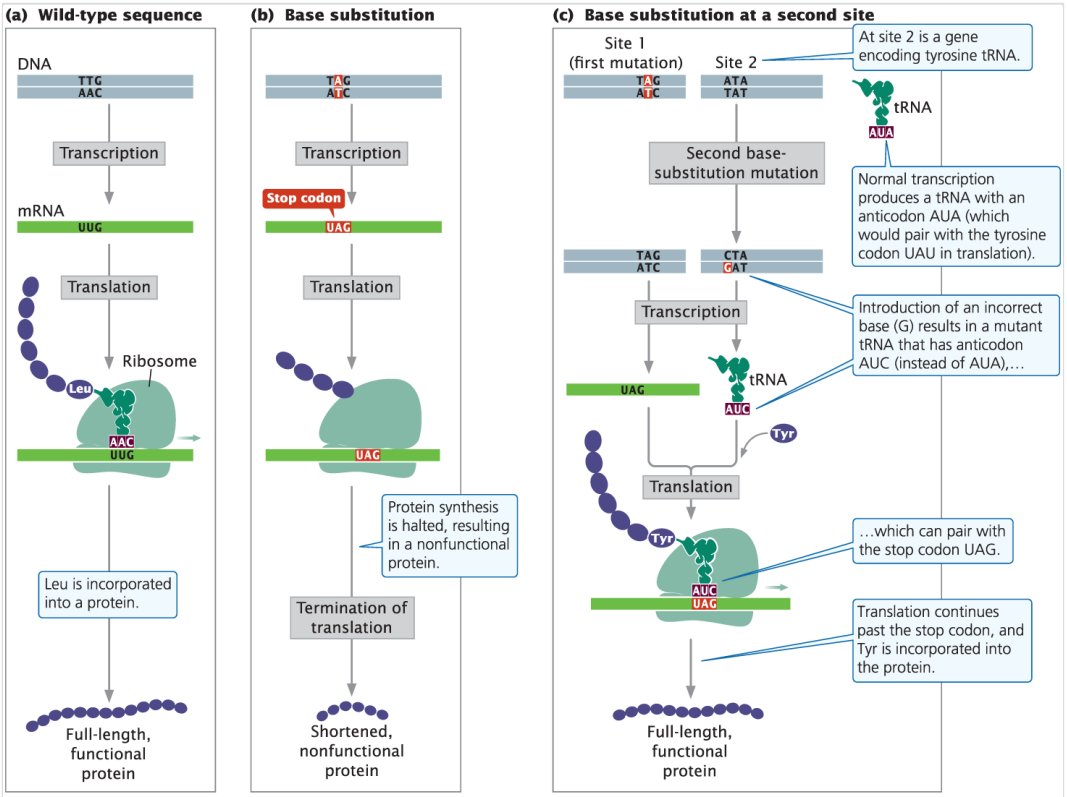
Similarly, a mutation due to an insertion may be suppressed by a subsequent deletion in the same gene.

A third way in which an intragenic suppressor mutation may work is by making compensatory changes in the protein. A first missense mutation can alter the folding of a polypeptide chain by changing the way in which amino acids in the protein interact with one another. A second missense mutation at a different site (the suppressor mutation) can re-create the original folding pattern by restoring the interactions between the amino acids.

INTERGENIC SUPPRESSOR MUTATIONS

An **intergenic suppressor mutation**, in contrast, occurs in a gene other than the one bearing the original mutation that it suppresses. These mutations, also known as *extragenic suppressor mutations*, sometimes work by changing the way the mRNA is translated. In the example illustrated in **Figure 18.9a**, the original DNA sequence is AAC (UUG in the mRNA) and specifies leucine. This sequence mutates to ATC (UAG in the mRNA), a stop codon (**Figure 18.9b**). The ATC nonsense mutation could be suppressed by a second mutation in a different gene that encodes a tRNA; this second mutation would result in a tRNA anticodon capable of pairing with the UAG stop codon (**Figure 18.9c**). For example, the gene that encodes the tRNA for tyrosine ($tRNA^{Tyr}$), which has the anticodon AUA, might be mutated to have the anticodon AUC, which would

then pair with the UAG stop codon. Instead of translation terminating at the UAG codon, tyrosine would be inserted into the protein, and a full-length protein would be produced, although tyrosine would now substitute for leucine. The effect of this change would depend on the role of this amino acid in the overall structure of the protein, but the effect of the suppressor mutation would probably be less detrimental than the effect of the nonsense mutation, which would halt translation prematurely.



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18.9 An intergenic suppressor mutation occurs in a gene other than the one bearing the original mutation that it suppresses. (a) A wild-type sequence produces a full-length, functional protein. (b) A base substitution at a site in that gene produces a premature stop codon, resulting in a truncated, nonfunctional protein. (c) A base substitution at a site in another gene, which in this case encodes tRNA, alters the anticodon of $tRNA^{Tyr}$; $tRNA^{Tyr}$ can then pair with the

stop codon produced by the original mutation, allowing tyrosine to be incorporated into the protein and translation to continue.

Description

Part a is labeled wild type sequence. A double D N A strand is present at the top. The sequence of the top strand is as follows: T, T, G. The sequence of the bottom strand is as follows: A, A, C. Transcription occurs and an m R N A is obtained below with the following sequence: U, U, G. Translation occurs and a ribosome with complementary bases at its t R N A (A, A, C) attaches to the m R N A bases U, U, G. A leucine is obtained from the ribosome. Text reads, Leu is incorporated into a protein. A full-length, functional protein is obtained below.

Part b is labeled wild base substitution. A double D N A strand is present at the top. The sequence of the top strand is as follows: T, A (highlighted), G. The sequence of the bottom strand is as follows: A, T (highlighted), C. Transcription occurs and an m R N A is obtained below with the following sequence: U, A, G. The sequence is labeled stop codon. Translation occurs and a ribosome attaches to the m R N A bases U, A, G. Translation is terminated. A caption reads, Protein synthesis is halted, resulting in a nonfunctional protein. A Shortened, nonfunctional protein is obtained below.

Part c is labeled Base substitution at a second site. A double D N A strand is present at the top left and is labeled site 1 (first mutation). The sequence of the top strand is as follows: T, A (highlighted), G. The sequence of the bottom strand is as follows: A, T (highlighted), C. Another double D N A strand is present to the right and is labeled site 2. A caption reads, At site 2 is a gene encoding tyrosine t R N A. The sequence of the top strand is as follows: A, T, A. The sequence of the bottom strand is as follows: T, A, T. A t R N A with bases A, U, A is present to the right. A caption reads, Normal transcription produces a t R N A with an anticodon A U A (which would pair with the tyrosine codon U A U in translation). Second base substitution Mutation occurs at site 2 and a D N A double strand is obtained below. The sequence of the top strand is as follows: C, T, A. The sequence of the bottom strand is as follows: G (highlighted), A, T. Transcription occurs for site 1 and base substituted D N A obtained from site 2. For site 1, the m R N A produced below has the following sequence: U, A, G. A t R N A with bases A, U, C is obtained from the base substituted D N A double strand after transcription. T y r enters the process and translation occurs for m R N A obtained and t R N A obtained. The ribosome with t R N A (A, U, C) attaches on the m R N A (U, A, G). A t y r amino acid is obtained from the ribosome. A full-length, functional

protein is obtained below. A caption reads, Introduction of an incorrect base (G) results in a mutant t R N A that has anticodon A U C (instead of A U A) which can pair with the stop codon U A G. A forward arrow indicates the ribosome will move. A caption reads, Translation continues past the stop codon, and T y r is incorporated into the protein.

Because cells in many organisms have multiple copies of tRNA genes, other nonmutated copies of $tRNA^{Tyr}$ would remain available to recognize tyrosine codons in mRNA transcripts. We might expect that the tRNAs encoded by the gene with the suppressor mutation just described would suppress the normal stop codons at the ends of other coding sequences as well as the one in the transcript of the original mutant gene, resulting in the production of longer-than-normal proteins, but this event does not usually take place.

Intergenic suppressor mutations can also work through gene interactions (see Chapter 5). For example, polypeptide chains that are produced by two different genes may interact to produce a functional protein. A mutation in one gene may alter the encoded polypeptide such that the interaction between the two polypeptides is destroyed, in which case a functional protein is not produced. A suppressor mutation in the second gene may produce a compensatory change in its polypeptide, therefore restoring the original interaction. Characteristics of some of the different types of mutations are summarized in **Table 18.2**.

TABLE 18.2 Characteristics of different types of mutations

Type of Mutation	Definition

Base substitution	
Transition	Base substitution in which a purine replaces a purine or a pyrimidine replaces a pyrimidine
Transversion	Base substitution in which a purine replaces a pyrimidine or a pyrimidine replaces a purine
Insertions and deletions (indels)	
Insertion	Addition of one or more nucleotides
Deletion	Deletion of one or more nucleotides
Frameshift mutation	Insertion or deletion that alters the reading frame of a gene
In-frame deletion or insertion	Deletion or insertion of a multiple of three nucleotides that does not alter the reading frame
Expanding nucleotide repeats	Repeated sequence of a set of nucleotides in which the number of copies of the sequence increases
Direction	
Forward mutation	Changes the wild-type phenotype to a mutant phenotype
Reverse mutation	Changes a mutant phenotype back to the wild-type phenotype
Effects	
Missense mutation	Changes a sense codon into a different sense codon, resulting in the incorporation of a different amino acid in the protein
Nonsense mutation	Changes a sense codon into a nonsense (stop) codon, causing premature termination of translation
Silent mutation	Changes a sense codon into a synonymous codon, leaving unchanged the amino acid sequence of the protein
Neutral mutation	Changes the amino acid sequence of a protein without altering its ability to function
Loss-of-function mutation	Causes a complete or partial loss of function

Gain-of-function mutation	Causes the appearance of a new trait or function or causes the appearance of a trait in inappropriate tissue or at an inappropriate time
Lethal mutation	Causes premature death
Suppression	
Suppressor mutation	Suppresses the effect of an earlier mutation at a different site
Intragenic suppressor mutation	Suppresses the effect of an earlier mutation within the same gene
Intergenic suppressor mutation	Suppresses the effect of an earlier mutation in another gene

CONCEPTS

A suppressor mutation overrides the effect of an earlier mutation at a different site. An intragenic suppressor mutation occurs within the *same* gene that contains the original mutation; an intergenic suppressor mutation occurs in a *different* gene.

✔ CONCEPT CHECK 2

How is a suppressor mutation different from a reverse mutation?

WORKED PROBLEM

A gene encodes a protein with the following amino acid sequence:

Met-Arg-Cys-Ile-Lys-Arg

A mutation of a single nucleotide alters the amino acid sequence to

Met-Asp-Ala-Leu-Lys-Gly-Glu-Ala-Pro-Val

A second single-nucleotide mutation occurs in the same gene and suppresses the effects of the first mutation (an intragenic suppressor). With the original mutation and the intragenic suppressor present, the protein has the following amino acid sequence:

Met-Asp-Gly-Ile-Lys-Arg

What is the nature and location of the first mutation and the intragenic suppressor mutation?

Solution Strategy

What information is required in your answer to the problem?

The nature and location of the first mutation and the intragenic suppressor mutation.

What information is provided to solve the problem?

- The amino acid sequence of the protein encoded by the original nonmutated gene.
- The amino acid sequence of the protein encoded by the mutated gene.
- The amino acid sequence of the protein encoded by the mutated gene and the intragenic suppressor.

Solution Steps

The first mutation alters the reading frame, because all amino acids after Met are changed, including the stop codon (which results in a longer protein). Insertions and deletions affect the reading frame; the original mutation consists of a single-nucleotide insertion or deletion in the second codon. The intragenic suppressor restores the reading frame; the intragenic suppressor is most likely a single-nucleotide insertion or deletion. If the first mutation is an insertion, the suppressor must be a deletion; if the first mutation is a deletion, then the suppressor must be an insertion. Notice that the protein produced by the suppressor still differs from the original protein at the second and third amino acids, but the second amino acid produced by the suppressor is the same as that in the protein produced by the original mutation. Thus, the suppressor mutation must have occurred in the third codon, because the suppressor does not alter the second amino acid.

» For more practice with analyzing mutations, try working **Problem 24** at the end of the chapter.

Mutation Rates

The frequency with which a wild-type allele at a locus changes into a mutant allele is referred to as the **mutation rate**. The mutation rate is generally expressed as the number of mutations per biological unit, which may be mutations per cell division, per gamete, or per round of replication. For example, achondroplasia is a type of hereditary dwarfism in humans that results from a dominant mutation. On average, about four achondroplasia mutations arise in every 100,000 gametes, and so the mutation rate is $^{4}/_{100,000}$, or 0.00004 mutations per gamete. The mutation rate provides information about how often a mutation arises.

FACTORS AFFECTING MUTATION RATES

Calculations of mutation rates are affected by three factors. First, they depend on the frequency with which changes in DNA take place. Mutations can arise as spontaneous molecular changes in DNA, or they can be induced by chemical, biological, or physical agents in the environment.

The second factor influencing the mutation rate is the probability that when an alteration in DNA takes place, it will be repaired. Most cells possess a number of mechanisms for repairing altered DNA (see Section 18.5), so most alterations are corrected before they are replicated. If these repair systems are effective, mutation rates will be low; if they are faulty, mutation rates will be elevated. Some mutations increase the overall rate of mutation at other genes; these

mutations usually occur in genes that encode components of the replication machinery or DNA-repair enzymes.

The third factor is the probability that a mutation will be detected. When DNA is sequenced, all mutations are potentially detectable. In practice, however, mutations are usually detected by their phenotypic effects. Some mutations may appear to arise at a higher rate simply because they are easier to detect.

VARIATION IN MUTATION RATES

We can draw several general conclusions about mutation rates, though they vary among genes and among species (Table 18.3). First, spontaneous mutation rates are low for all organisms studied. Typical mutation rates for bacterial genes range from about 1 to 100 mutations per 10 billion cells (from 1×10^{-8} to 1×10^{-10}). The mutation rates for most eukaryotic genes are a bit higher, from about 1 to 10 mutations per million gametes (from 1×10^{-5} to 1×10^{-6}). These higher values in eukaryotes may be due to the fact that the rates are calculated *per gamete*, and that several cell divisions are required to produce a gamete, whereas mutation rates in prokaryotic cells are calculated *per cell division*.

TABLE 18.3 Mutation rates of different genes in different organisms

Organism	Mutation	Rate	Unit
Influenza A	Nonstructural genes		Per replication

		2.0×10^{-6}	
Bacteriophage T2	Lysis inhibition Host range	1×10^{-8} 3×10^{-9}	Per replication
Escherichia coli	Lactose fermentation Histidine requirement	2×10^{-7} 2×10^{-8}	Per cell division
Neurospora crassa	Inositol requirement Adenine requirement	8×10^{-8} 4×10^{-8}	Per asexual spore
Corn	Kernel color	2.2×10^{-6}	Per gamete
Drosophila	Eye color Allozymes	4×10^{-5} 5.14×10^{-6}	Per gamete
Mouse	Albino coat color Dilution coat color	4.5×10^{-5} 3×10^{-5}	Per gamete
Human	Huntington disease Achondroplasia	1×10^{-6} 1×10^{-5}	Per gamete
	Neurofibromatosis (Michigan)	1×10^{-4}	
	Hemophilia A (Finland)	3.2×10^{-5}	
	Duchenne muscular dystrophy (Wisconsin)	9.2×10^{-5}	

The differences in mutation rates among species may be due to differing abilities to repair mutations, unequal exposures to mutagens, or biological differences in rates of spontaneous mutations. Even within a single species, spontaneous mutation rates vary among genes. The reason for this variation is not entirely

understood, but some regions of DNA are known hotspots for mutations.

Recent research suggests that fewer mutations occur in DNA sequences that are associated with nucleosomes (see Chapter 11). Reduced mutation rates may occur in these sequences because DNA associated with nucleosomes is less exposed to mutagens, but they could also be explained by the effect of nucleosomes on DNA repair, recombination, or replication, all of which influence the rate of mutation.

Several recent studies have measured mutation rates directly by sequencing genes of organisms over a number of generations. These new studies suggest that mutation rates are often higher than those previously measured on the basis of changes in phenotype. In one study, geneticists sequenced randomly chosen stretches of DNA in the nematode *Caenorhabditis elegans* and found about 2.1 mutations per genome per generation, which was 10 times higher than previous estimates based on phenotypic changes. The researchers found that about half of the mutations were insertions and deletions.

Recent genome sequencing has also provided more accurate information about mutation rates in humans. Several sequencing studies suggest that the overall rate of base substitutions in humans is about 1×10^{-8} mutations per base pair per generation. Other

research suggests that each person carries approximately 100 new loss-of-function germ-line mutations.

THINK-PAIR-SHARE Question 3

ADAPTIVE MUTATION

As will be discussed in Chapters 24 through 26, genetic variation is critical for evolutionary change that brings about adaptation to new environments. New genetic variants arise primarily through mutation. For many years, genetic variation was assumed to arise randomly and at rates that are independent of the need for adaptation. However, some evidence suggests that stressful environments—where adaptation may be necessary to survive—can induce more mutations in bacteria, a process that has been termed **adaptive mutation**. The idea of adaptive mutation has been intensely debated; critics counter that most mutations are expected to be deleterious, and therefore increased mutagenesis would probably be harmful most of the time.

CONCEPTS

The mutation rate is the frequency with which a specific mutation arises. Rates of mutations are generally low and are affected by environmental and genetic factors.

✔ CONCEPT CHECK 3

What three factors affect mutation rates?

18.2 Mutations May Be Caused by a Number of Different Factors

Mutations result from both internal and external factors. Those that occur under normal conditions are termed **spontaneous mutations**, whereas those that result from changes caused by environmental chemicals or radiation are **induced mutations**.

Spontaneous Replication Errors

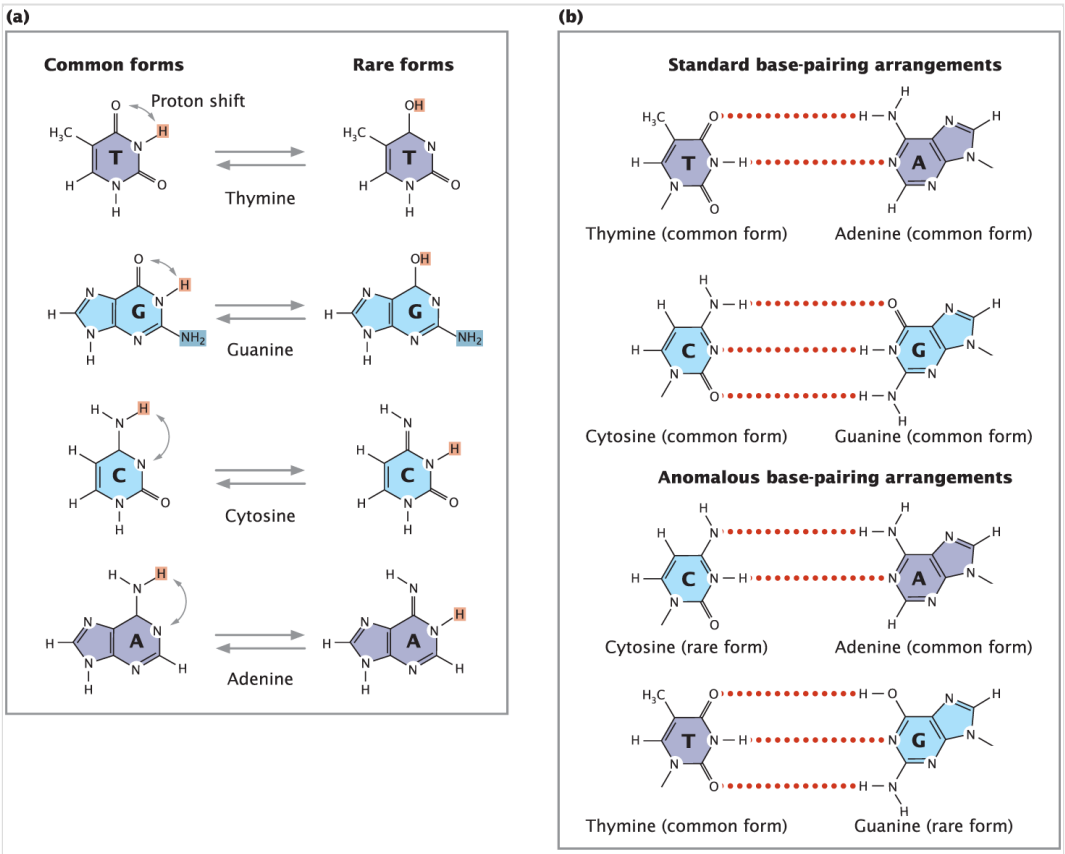
Replication is amazingly accurate: less than one error in a billion nucleotides arises in the course of DNA synthesis (see Chapter 12). However, spontaneous replication errors do occasionally occur.

THINK-PAIR-SHARE Question 4

TAUTOMERIC SHIFTS

In their 1953 paper describing DNA structure, Watson and Crick (see Chapter 10) originally proposed that the primary cause of spontaneous replication errors is tautomeric shifts, in which the positions of protons (hydrogen atoms) in the DNA bases change. Each of the four DNA bases exists in different chemical forms, called *tautomers* (**Figure 18.10a**). The two tautomeric forms of each base

are in dynamic equilibrium, although one form is much more common than the other. The standard Watson-and-Crick base pairings—adenine with thymine, and cytosine with guanine—occur between the common forms of the bases, but if the bases are in their rare tautomeric forms, other base pairings are possible (Figure 18.10b). For example, the common form of cytosine pairs with guanine, but the rare tautomer of cytosine pairs with adenine.



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18.10 Purine and pyrimidine bases exist in different forms called tautomers. (a) A tautomeric shift takes place when a proton changes its position, resulting in a rare tautomeric form. (b) Standard and anomalous base-pairing arrangements that arise if bases are in the rare tautomeric forms.

Description

Part a on the left shows common and rare forms of thymine, Guanine, Cytosine, and Adenine.

For Thymine:

A tautomeric shift in a common form of thymine results in a rare form of thymine.

The common form of thymine has C 5 bonded to a methyl group. The hydrogen atom bonded to N 1 is highlighted. A proton shift takes place from N 1 to the oxygen atom at C 6. The rare form of thymine has a similar structure as that of common form, except that C 6 is now bonded to a hydroxyl group, in which the hydrogen atom is highlighted.

For Guanine:

A tautomeric shift in a common form of guanine results in a rare form of guanine.

The common form of guanine has C 2 bonded to amino group. A proton shift takes place from N 1 to carbonyl group of C 6. The rare form of guanine has a similar structure as that of common form, except that C 6 is now bonded to a hydroxyl group, in which hydrogen atom is highlighted.

For Cytosine:

A tautomeric shift in a common form of cytosine results in a rare form of cytosine.

The common form of cytosine has C 4 bonded to amino group. The hydrogen atom bonded to N 1 is highlighted. A proton shift takes place from the amino group at C 4 to N 3. The rare form of cytosine has a similar structure as that of common form, except that N 3 is now bonded to a hydrogen atom, in which the hydrogen atom is highlighted.

For Adenine:

An illustration shows a tautomeric shift in a common form of adenine, resulting in a rare form of adenine.

The common form of adenine has C 6 bonded to an amino group, in which one of the two hydrogen atoms is highlighted. A proton shift takes place from the amino group at C 6 to N 1. The rare form of adenine has a similar structure as that of common form, except that N 1 is now bonded to a hydrogen atom, highlighted and C 6 is double bonded to an N H group.

Part b on the right shows Standard base-pairing arrangements and anomalous base-pairing arrangements.

Standard base-pairing arrangements show:

Thymine (common form) and adenine (common form) show double bonds where hydrogen atom of N 1 of thymine is bonded to N 1 of adenine. The oxygen atom at C 6 is bonded to one of the hydrogen atoms of amino group at C 6 of adenine.

Cytosine (common form) and Guanine (common form) show triple bonds. One of the H atoms from amino group at C 4 of cytosine is bonded to carbonyl O atom at C 6 of guanine. N 3 atom of cytosine is bonded to H atom at N 1 of guanine. One carbonyl O at C 2 of cytosine is bonded to one of the H atom of amino group at C 2 of guanine.

Anomalous base-pairing arrangements show:

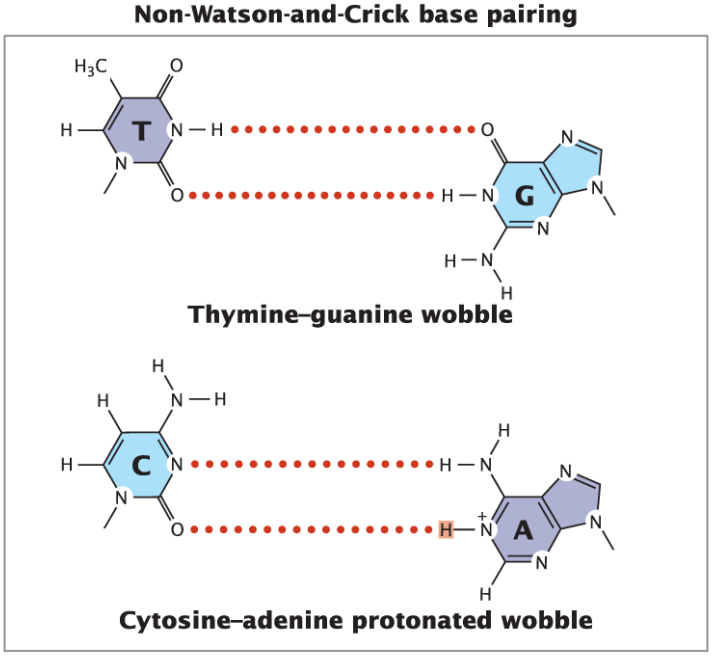
Cytosine (rare form) and adenine (common form) show double bonds. N atom of the N H group at C 4 of cytosine is bonded to one of the H atom of amino group at C 6 of adenine. H atom bonded to N 3 of cytosine is bonded to N 1 of adenine.

Thymine (common form) and Guanine (rare form) show triple bonds. One of the carbonyl O at C 4 of thymine is bonded to H atom of hydroxyl group at C 6 of guanine. One of the H atoms of N 3 of thymine is bonded to N 1 of guanine. One of the carbonyl O of thymine is bonded to one of the H atom of amino group at C 2 of guanine.

Although Watson and Crick proposed that tautomeric shifts might produce mutations, tautomeric forms of the bases were difficult to detect in DNA, and evidence of their role in mutation was lacking. Many researchers assumed that other structures, such as protonated bases (see the following discussion), were responsible for base mispairing during replication. Recently, however, researchers have detected base mispairings in DNA that involve rare base tautomers.

MISPAIRING DUE TO OTHER STRUCTURES

Mispairings between bases in DNA can arise through wobble (see Chapter 15), in which normal, protonated, and other forms of the bases are able to pair because of flexibility in the DNA helical structure (**Figure 18.11**). These structures have been detected in DNA molecules and are responsible for some mispairings in replication.



18.11 Nonstandard base pairings can occur as a result of the flexibility in DNA structure. Thymine and guanine in their normal forms can pair through wobble. Cytosine and adenine can pair through wobble when adenine is protonated (has an extra hydrogen atom).

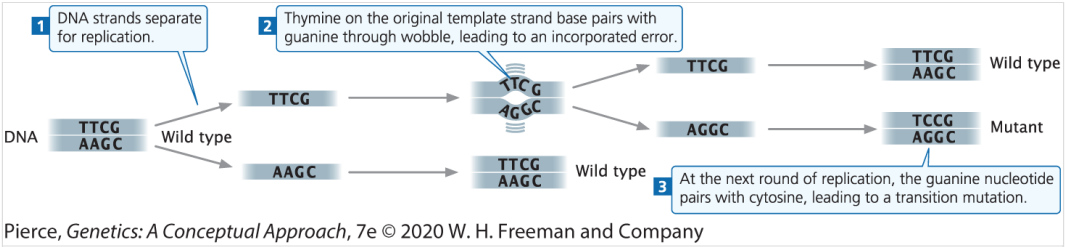
Description

Thymine-guanine wobble shows a double bond between thymine and guanine. One of the H atom at N 3 of thymine is bonded to carbonyl O of guanine. The O bonded to C 2 of thymine is bonded to an H atom of N 1 of guanine.

Cytosine-adenine protonated wobble shows a double bond. N 3 of Cytosine is bonded to one of the H atoms of amino group at C 6 of adenine. The O atom bonded to C 2 of cytosine is bonded to the H atom of N 1, which carries a positive change, of adenine.

INCORPORATED ERRORS AND REPLICATED ERRORS

When a base substitution causes a mispaired base to be incorporated into a newly synthesized nucleotide chain, an **incorporated error** is said to have occurred. Suppose that in replication, thymine (which normally pairs with adenine) mispairs with guanine through wobble (**Figure 18.12**). In the next round of replication, the two mismatched bases separate, and each serves as a template for the synthesis of a new nucleotide strand. This time, thymine pairs with adenine, producing another copy of the original DNA sequence. On the other strand, however, the incorrectly incorporated guanine serves as the template and pairs with cytosine, producing a new DNA molecule that has an error: a C • G pair in place of the original T • A pair (a T • A → C • G base substitution). The original incorporated error (the T–G mispairing) leads to a **replicated error** (the C • G base pair instead of the original T • A base pair), which creates a permanent mutation because all the base pairings are correct and there is no way for repair systems to detect the error.



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18.12 Wobble base pairing may lead to a replicated error.

Description

The illustration shows a D N A double strand. The sequence of the top strand is as follows: T, T, C, G. The sequence of the bottom strand is as follows: A, A, G, C. The strands separate and a caption reads, D N A strands separate for replication. A forward arrow divides into two on the right.

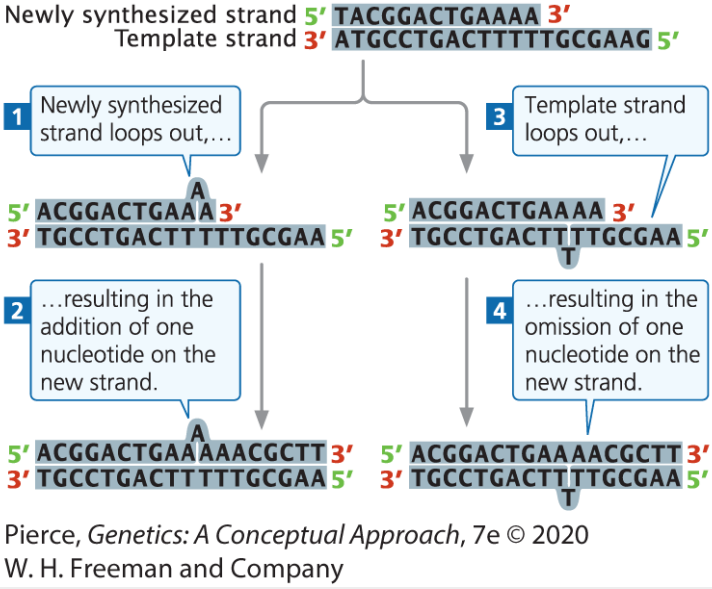
The arrow on the top leads to a strand with the following sequence: T, T, C, G. It further leads to a double strand. The top strand reads the following: T, T, C, G. The complementary bottom strand is: A, G, G, C. The nucleotides in the center bulge outside. A caption reads, Thymine on the original template strand base pairs with guanine through wobble, leading to an incorporated error. Both the strands separate. A forward arrow from the strands divides into two. The top strand now (T, T, C, G) further changes to the wild type D N A double strand with T, T, C, G at the top strand and A, A, G, C at the bottom strand. The bottom strand (A, G, G, C) further changes to mutant type D N A double strand with T, C, C, G at the top strand and A, G, G, C at the bottom strand. A caption reads, At the next round of replication, the guanine nucleotide pairs with cytosine, leading to a transition mutation.

The arrow at the bottom leads to strand A A G C, that further leads to a wild type D N A double strand with T T C G at top strand and A A G C at bottom strand.

CAUSES OF DELETIONS AND INSERTIONS

Small insertions and deletions can arise spontaneously in replication and crossing over. Strand slippage can occur when one nucleotide

strand forms a small loop (Figure 18.13). If the looped-out nucleotides are on the newly synthesized strand, an insertion results. At the next round of replication, the insertion will be replicated, and both strands will contain the insertion. If the looped-out nucleotides are on the template strand, then the newly replicated strand will have a deletion, and this deletion will be perpetuated in subsequent rounds of replication.



18.13 Insertions and deletions may result from strand slippage.

Description

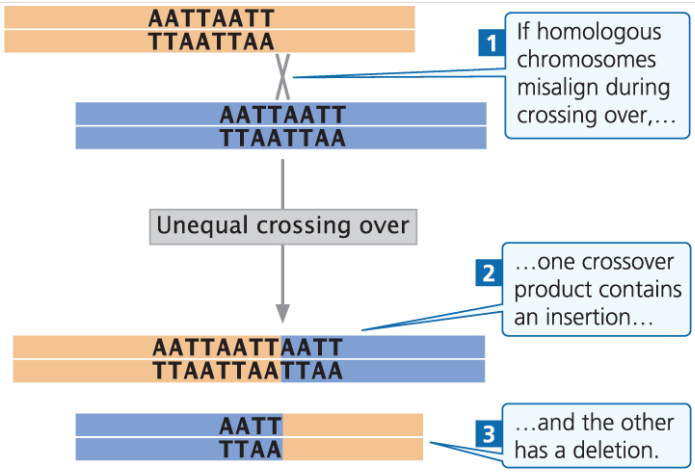
The illustration shows a newly synthesized strand with the following sequence: 5 prime-T, A, C, G, G, A, C, T, G, A, A, A, A-3 prime. Template strand below has the following sequence: 3 prime-A, T, G, C, C, T, G, A, C, T, T, T, T, T, G, C, G, A, A ,G-5 prime. A downward arrow divides into two arrows below.

The arrow on the left shows last second nucleotide of the newly synthesized strand loops out. The top strand now is as follows: A, C, G, G, A, C, T, G, A, A, A (looped out), A. The bottom strand is as follows: T, G, C, C, T, G, A, C, T, T, T, T, T, G, C, G, A, A. It then leads to a double strand below. The top strand now is as follows: A, C, G, G, A, C, T, G, A, A, A

(looped out), A, A, A, C, G, C, T, T. The bottom strand is as follows: T, G, C, C, T, G, A, C, T, T, T, T, T, G, C, G, A, A. A caption reads, Newly synthesized strand loops out resulting in the addition of one nucleotide on the new strand.

The arrow on the right shows a T nucleotide on the template strand loops out. The top strand now is as follows: A, C, G, G, A, C, T, G, A, A, A, A. The bottom strand is as follows: T, G, C, C, T, G, A, C, T, T, T (looped out), T, T, G, C, G, A, A. The top strand now is as follows: A, C, G, G, A, C, T, G, A, A, A, A, C, G, C, T, T. The bottom strand is as follows: T, G, C, C, T, G, A, C, T, T, T (looped out), T, T, G, C, G, A, A. A caption reads, Template strand loops out. This further leads to a D N A double strand below resulting in the omission of one nucleotide on the new strand.

Another process that produces insertions and deletions is unequal crossing over (see Section 8.2). In normal crossing over, the homologous sequences of the two DNA molecules align, and crossing over produces no net change in the number of nucleotides in either molecule. Misaligned pairing, however, can cause unequal crossing over, which results in one DNA molecule with an insertion and the other with a deletion (**Figure 18.14**).



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18.14 Unequal crossing over produces insertions and deletions.

Description

The illustration shows two pairs of rod-shaped homologous chromosomes at the top. The first pair, orange-colored, at the top has the following sequence: Top chromosome: A, A, T, T, A, A, T, T; Bottom chromosome: T, T, A, A, T, T, A, A.

The second pair, blue colored, below has the following sequence: Top chromosome: A, A, T, T, A, A, T, T; Bottom chromosome: T, T, A, A, T, T, A, A.

Unequal crossing over takes place.

The first longer, rod shaped chromosome pair obtained below has both orange and blue colored regions.

The top chromosome obtained has the following sequence: Orange: A, A, T, T, A, A, T, T; blue: A, A, T, T.

The bottom chromosome obtained has the following sequence: Orange: T, T, A, A, T, T, A, A; blue: T, T, A, A.

The second short, rod shaped chromosome pair obtained below has both orange and blue colored regions.

The top chromosome obtained has the following sequence: Blue: A, A, T, T; orange: no nucleotides present.

The bottom chromosome obtained has the following sequence: Blue: T, T, A, A; orange: no nucleotides present.

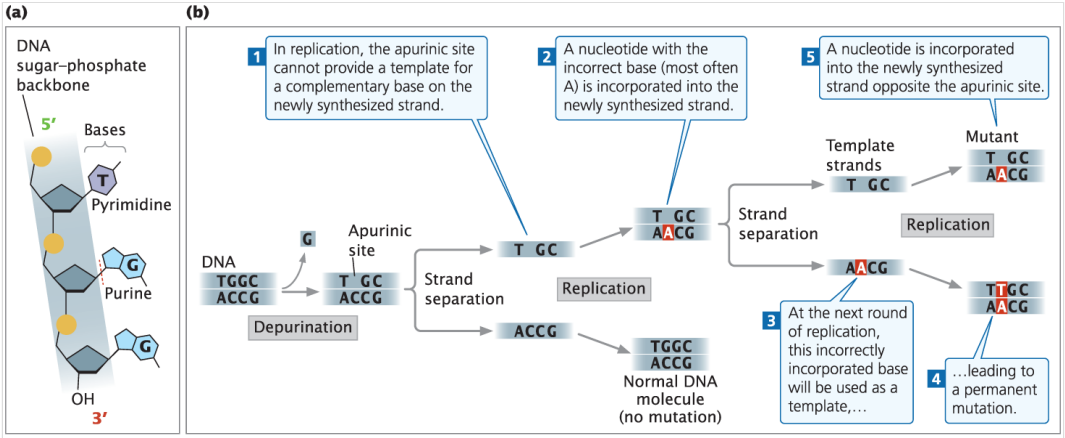
Captions throughout read, If homologous chromosomes misalign during crossing over, one crossover product contains an insertion and the other has a deletion.

CONCEPTS

Spontaneous replication errors arise from altered base structures and from wobble. Small insertions and deletions can occur through strand slippage during replication and through unequal crossing over.

Spontaneous Chemical Changes

In addition to spontaneous mutations that arise in replication, mutations also result from spontaneous chemical changes in DNA. One such change is **depurination**, the loss of a purine base from a nucleotide. Depurination results when the covalent bond connecting the purine to the 1′-carbon atom of the deoxyribose sugar breaks (Figure 18.15a), producing an apurinic site, a nucleotide that lacks its purine base. An apurinic site cannot act as a template for a complementary base in replication. In the absence of base-pairing constraints, an incorrect nucleotide (most often adenine) is incorporated into the newly synthesized DNA strand opposite the apurinic site (Figure 18.15b), frequently leading to an incorporated error. The incorporated error is then transformed into a replicated error at the next round of replication. Depurination is a common cause of spontaneous mutation; a mammalian cell in culture loses approximately 10,000 purines every day. Loss of pyrimidine bases also occurs, but at a much lower rate than depurination.



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18.15 Depurination (the loss of a purine base from a nucleotide) may lead to a base substitution. (a) Depurination occurs when the covalent bond connecting a purine to the 1′ carbon of the sugar is broken (indicated by dotted red line). (b) Replication of a template strand with an apurinic site may lead to an incorporated error.

Description

Part a on the left shows a D N A sugar phosphate backbone running from 5 prime at the top to 3 prime at the bottom. The pentose sugars have bases on the C 1 and phosphate molecules on the C 4. The first sugar molecule at the top has pyrimidine T attached to it. The sugar below it has purine G attached. The bond that joins purine and sugar is dissected by a red, vertical dotted line. The sugar below has purine G attached.

Part b on the right shows a D N A double strand on the left side. The sequence of the top strand is as follows: T, G, G, C. The sequence of the bottom strand is as follows: A, C, C, G. One G nucleotide from the top strand gets separated, indicating depurination. A forward arrow leads to the D N A double strand obtained. The sequence of the top strand is as follows: T, apurinic site, G, C. The sequence of the bottom strand is as follows: A, C, C, G. Strand separation occurs and is represented by a forward arrow dividing into two arrows.

The arrow at the top leads to the strand with the following sequence: T, blank, G, C. A caption reads, In replication, the apurinic site cannot provide a template for a complementary base on the newly synthesized strand. It further leads to a double strand. The sequence of the top strand is as follows: T, blank, G, C. The sequence of the bottom strand is as follows: A, A (highlighted), C, G. A caption reads, A nucleotide with the incorrect base (most often A) is incorporated into the newly synthesized strand. Strand

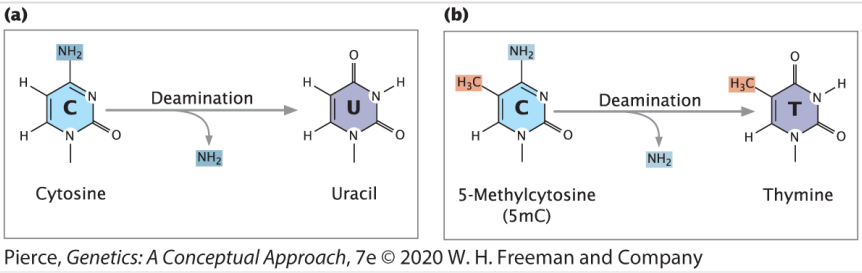
separation occurs and is represented by a forward arrow that divides into two. The arrow at the top leads to template strands: T, blank, G, C. Replication occurs and a mutant D N A double strand is obtained. The top strand has the following sequence: T, blank, G, C. The bottom strand has the following sequence: A, A (highlighted), C, G. A caption reads, A nucleotide is incorporated into the newly synthesized strand opposite the apurinic site.

The arrow at the bottom leads to the following strand: A, A (highlighted), C, G. Replication occurs and a D N A double strand is obtained. The top strand has the following sequence: T, T (highlighted), G, C. The bottom strand has the following sequence: A, A (highlighted), C, G. A caption reads, At the next round of replication, this incorrectly incorporated base will be used as a template, leading to a permanent mutation.

The arrow at the bottom, after first strand separation, leads to the following strand: A, C, C, G. Replication occurs and a D N A double strand is obtained on the right. The top strand has the following sequence: T, G, G, C. The bottom strand has the following sequence: A, C, C, G. Text below reads, Normal D N A molecule (no mutation).

Another spontaneously occurring chemical change that takes place in DNA is **deamination**, the loss of an amino group (NH_2) from a base. Deamination may be spontaneous or may be induced by mutagenic chemicals. Deamination can alter the pairing properties of a base: the deamination of cytosine, for example, produces uracil (**Figure 18.16a**), which pairs with adenine in replication. After another round of replication, the adenine will pair with thymine, creating a T • A pair in place of the original C • G pair (C • G → U • A → T • A); this chemical change is a transition mutation. This type of mutation is usually prevented by enzymes that remove uracil whenever it is found in DNA. The ability of these enzymes to recognize the product of cytosine deamination may explain why thymine, not uracil, is found in DNA. In mammals, including humans, some cytosine bases in DNA are naturally methylated and

exist in the form of 5-methylcytosine (5mC) (see Figure 10.20). When deaminated, 5mC becomes thymine (**Figure 18.16b**). Because thymine pairs with adenine in replication, the deamination of 5-methylcytosine changes an original C • G pair to T • A (C • G → 5mC • G → T • G → T • A). Consequently, C • G → T • A transitions are frequent in mammalian cells, and 5mC sites are mutation hotspots in humans. **› TRY PROBLEM 28**



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18.16 Deamination alters DNA bases.

Description

The part a on the left shows cytosine with an amino group on its C 4 (highlighted in green). A forward arrow represents deamination where an amino group is removed. Uracil is obtained on the right.

The part b on the right shows 5 methyl cytosine (5 m C) which has a methyl group at C 5 (highlighted in red) and an amino group C 4 (highlighted in green). A forward arrow represents deamination where an amino group is removed. Thymine is obtained on the right. The methyl group at its C 5 is highlighted in red.

CONCEPTS

Some mutations arise from spontaneous alterations in DNA structure, such as depurination and deamination, which can alter the pairing properties of the bases and cause errors in subsequent rounds of replication.

Chemically Induced Mutations

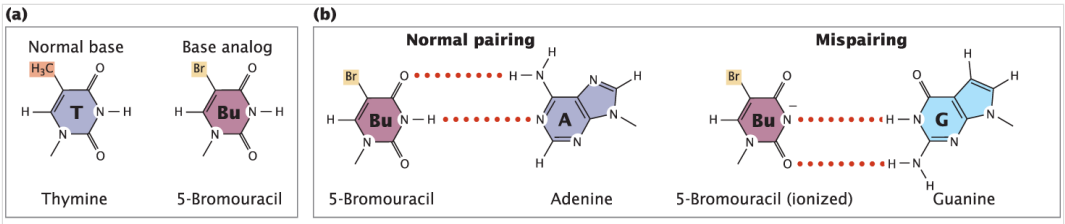
Although many mutations arise spontaneously, a number of environmental agents are capable of damaging DNA, including certain chemicals and radiation. Any environmental agent that significantly increases the rate of mutation above the spontaneous rate is called a **mutagen**.

The first discovery of a chemical mutagen was made by Charlotte Auerbach, who started her career in Berlin researching the development of mutants in *Drosophila*. Faced with increasing anti-Semitism in Nazi Germany, Auerbach immigrated to Britain in 1933. There she continued her research on *Drosophila* and, in 1940, began a collaboration with pharmacologist John Robson at the University of Edinburgh on the mutagenic effects of mustard gas, which had been used as a chemical weapon in World War I. The experimental conditions were crude: they heated liquid mustard gas over a Bunsen burner on the roof of the pharmacology building and exposed the flies to the gas in a large chamber. After developing serious burns on her hands from the gas, Auerbach let others carry out the exposures, and she analyzed the flies. Auerbach and Robson showed that mustard gas is indeed a powerful mutagen, reducing the viability of gametes and increasing the numbers of mutations seen in the offspring of exposed flies. Because the research was part of a secret

project during World War II, publication of their findings was delayed until 1947.

BASE ANALOGS

One class of chemical mutagens consists of **base analogs**, chemicals with structures similar to those of any of the four standard nitrogenous bases of DNA. DNA polymerases cannot distinguish these analogs from the standard bases, so if base analogs are present during replication, they may be incorporated into newly synthesized DNA molecules. For example, 5-bromouracil (5BU) is an analog of thymine; it has the same structure as thymine except that it has a bromine (Br) atom on the 5-carbon atom instead of a methyl group (Figure 18.17a). Normally, 5BU pairs with adenine just as thymine does, but it occasionally mispairs with guanine (Figure 18.17b), leading to a transition (T • A → 5BU • A → 5BU • G → C • G), as shown in Figure 18.18. Through mispairing, 5BU can also be incorporated into a newly synthesized DNA strand opposite guanine. In the next round of replication, 5BU pairs with adenine, leading to another transition (G • C → G • 5BU → A • 5BU → A • T).



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18.17 5-Bromouracil (a base analog) resembles thymine, except that it has a bromine atom in place of a methyl group on the 5-carbon atom. Because of the

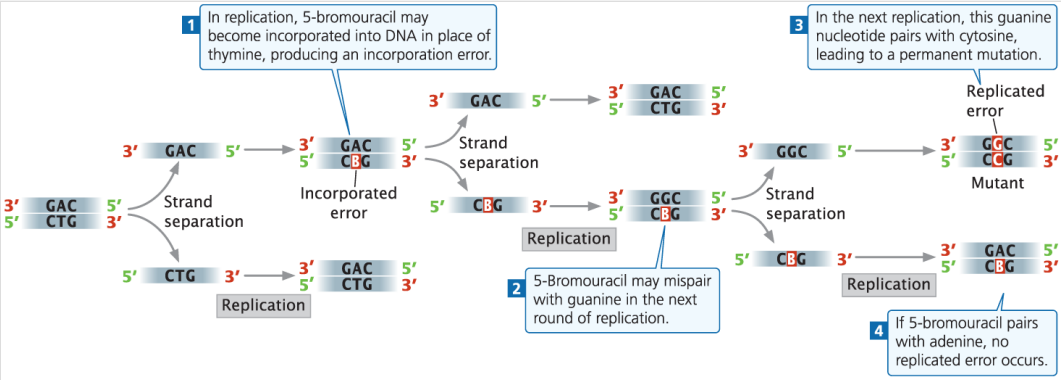
similarity in their structures, 5-bromouracil may be incorporated into DNA in place of thymine. Like thymine, 5-bromouracil normally pairs with adenine, but when ionized, it may pair with guanine through wobble.

Description

The part a on the left shows normal base thymine with a methyl group at C 5 highlighted. 5 bromouracil which is a base analog shows B r group at C 5 highlighted.

The part b on the right shows a normal pairing between 5 bromouracil and adenine. The carbonyl O at C 4 of 5 bromouracil is bonded to one of the H atom of amino group at C 6 of adenine. One of the H atoms of N 3 of 5 bromouracil is bonded to N 1 of adenine.

Mispairing between 5-Bromouracil (ionized) and guanine is shown on the right. N 3 with a negative charge of 5 bromouracil is bonded to H atom of N 1 of guanine. Carbonyl O bonded to C 2 is bonded to H atom of amino group at C 2 of guanine.



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18.18 Incorporation of 5-bromouracil into a DNA strand can lead to a replicated error.

Description

The illustration shows a D N A double strand on the left. The sequence of the top strand is as follows: 3 prime G, A, C 5 prime. The sequence of the bottom strand is as follows: 5 prime C, T, G 3 prime. Two curved forward arrows represent strand separation.

The arrow at the top leads to the following strand with sequence: G, A, C. It further leads to a D N A double strand. The sequence of the top strand is as follows: G, A, C. The sequence of the bottom strand is as follows: C, B (highlighted), G. B is labeled, incorporated error. A caption reads, In replication, 5-bromouracil may become incorporated into D N A in place of thymine, producing an incorporation error. A forward arrow dividing into two parts indicates strand separation. The top arrow leads to the following strand: G, A, C. This further leads to a D N A double strand. The sequence of the top strand is as follows: G, A, C. The sequence of the bottom strand is as follows: C, T, G. The bottom arrow leads to the following strand: C, B (highlighted), G. Replication occurs and a D N A double strand is obtained. The sequence of the top strand is as follows: G, G, C. The sequence of the bottom strand is as follows: C, B (highlighted), G. A caption reads, 5-Bromouracil may mispair with guanine in the next round of replication. A forward arrow dividing into two parts indicates strand separation. The top arrow leads to the following strand: G, G, C that further leads to a mutant D N A double strand with replicated errors. The sequence of the top strand is as follows: G, G (highlighted), C. The sequence of the bottom strand is as follows: C, C (highlighted), G. A caption reads, In the next replication, this guanine nucleotide pairs with cytosine, leading to a permanent mutation. The bottom arrow leads to the following strand: C, B (highlighted), G. Replication occurs and a D N A double strand is obtained. The sequence of the top strand is as follows: G, A, C. The sequence of the bottom strand is as follows: C, B (highlighted), G. A caption reads, If 5-bromouracil pairs with adenine, no replicated error occurs.

The arrow at the bottom, after first strand separation, leads to the following strand: C, T, G. Replication occurs and a D N A double strand is obtained. The sequence of the top strand is as follows: G, A, C. The sequence of the bottom strand is as follows: C, T, G.

A caption below reads, Conclusion: Incorporation of bromouracil followed by mispairing leads to a T A to C G transition mutation.

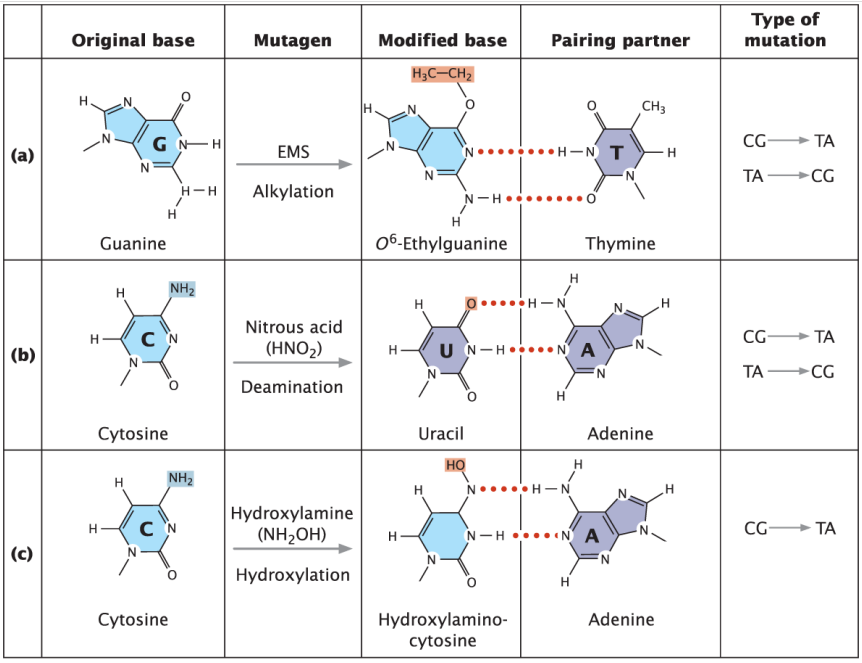
Another mutagenic chemical is 2-aminopurine (2AP), which is a base analog of adenine. Normally, 2AP pairs with thymine, but it may mispair with cytosine, causing a transition (T • A → T • 2AP → C • 2AP → C • G). Alternatively, 2AP may be incorporated through mispairing

into the newly synthesized DNA opposite cytosine and then later pair with thymine, leading to a C • G → C • 2AP → T • 2AP → T • A transition.

In the laboratory, mutations caused by base analogs can be reversed by treatment with the same analog or by treatment with a different analog.

ALKYLATING AGENTS

Alkylating agents are chemicals that donate alkyl groups, such as methyl (CH_3) and ethyl (CH_3—CH_2) groups, to nucleotide bases. For example, ethylmethylsulfonate (EMS) adds an ethyl group to guanine, producing O^6-ethylguanine, which pairs with thymine (**Figure 18.19a**). Thus, EMS produces C • G → T • A transitions. EMS is also capable of adding an ethyl group to thymine, producing 4-ethylthymine, which then pairs with guanine, leading to a T • A → C • G transition. Because EMS produces both C • G → T • A and T • A → C • G transitions, mutations produced by EMS can be reversed by additional treatment with EMS. Mustard gas is another alkylating agent.



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18.19 Chemicals may alter DNA bases. Shown here are some examples of mutations produced by chemical agents.

Description

The table has 3 rows and 5 columns. The column headers are as follows: Original base, Mutagen, Modified base, pairing partner, and type of mutation. The row entries are as follows:

Row 1: Original base: Guanine; Mutagen: E M S alkylation; Modified base and pairing partner: ethyl group bonded to O atom at C 6 is highlighted in O superscript 6 ethyl guanine, N 3 of O superscript 6 ethyl guanine is bonded to H atom of N 1 of thymine, one of the H atom of amino group at C 2 of O superscript 6 ethyl guanine is bonded to carbonyl O at C 2 of thymine; type of mutation: C G converts to T A and T A converts to C G.

Row 2: Original base: Cytosine with amino group at C 5 highlighted; Mutagen: Nitrous acid (H N O superscript 2) Deamination; Modified base and pairing partner: Carbonyl O at C 4 of uracil is bonded to one of the H atom of amino group at C 6 of adenine, H atom of N 3 of uracil is bonded to N 1 of adenine; type of mutation: C G converts to T A and T A converts to C G.

Row 3: Original base: Cytosine with amino group at C 5 highlighted; Mutagen: Hydroxylamine (N H subscript 2 O H) Hydroxylation; Modified base and pairing partner: N atom at C 4 of Hydroxyl aminocytosine has a hydroxyl group attached (highlighted) and is bonded to one of the H atom of amino group at C 6 of adenine, H atom of N 3 of Hydroxyl aminocytosine is bonded to N 1 of adenine; type of mutation: C G converts to T A.

DEAMINATING CHEMICALS

In addition to its spontaneous occurrence (see Figure 18.16), deamination can be induced by some chemicals. For instance, nitrous acid deaminates cytosine, creating uracil, which in the next round of replication pairs with adenine (**Figure 18.19b**), producing a C • G → T • A transition mutation. Nitrous acid also changes adenine into hypoxanthine, which pairs with cytosine, leading to a T • A → C • G transition. And nitrous acid also deaminates guanine, producing xanthine, which pairs with cytosine just as guanine does; however, xanthine can also pair with thymine, leading to a C • G → T • A transition. Nitrous acid produces exclusively transition mutations, and because both C • G → T • A and T • A → C • G transitions are produced, these mutations can be reversed with nitrous acid.

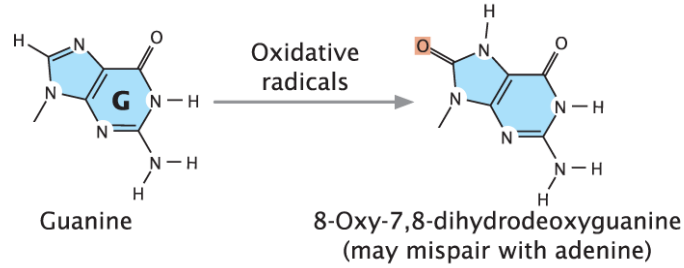
HYDROXYLAMINE

Hydroxylamine is a very specific base- modifying mutagen that adds a hydroxyl group to cytosine, converting it into hydroxylaminocytosine (**Figure 18.19c**). This conversion increases

the frequency of a rare tautomer that pairs with adenine instead of guanine and leads to C • G → T • A transitions. Because hydroxylamine acts only on cytosine, it does not generate T • A → C • G transitions; thus, hydroxylamine will not reverse the mutations that it produces. ❯ **TRY** **PROBLEM 26**

OXIDATIVE RADICALS

Reactive forms of oxygen (including superoxide radicals, hydrogen peroxide, and hydroxyl radicals) are produced in the course of normal aerobic metabolism as well as by radiation, ozone, peroxides, and certain drugs. These reactive forms of oxygen damage DNA and induce mutations by bringing about chemical changes in DNA. For example, oxidation converts guanine into 8-oxy-7,8-dihydrodeoxyguanine (**Figure 18.20**), which frequently mispairs with adenine instead of cytosine, causing a G • C → T • A transversion.



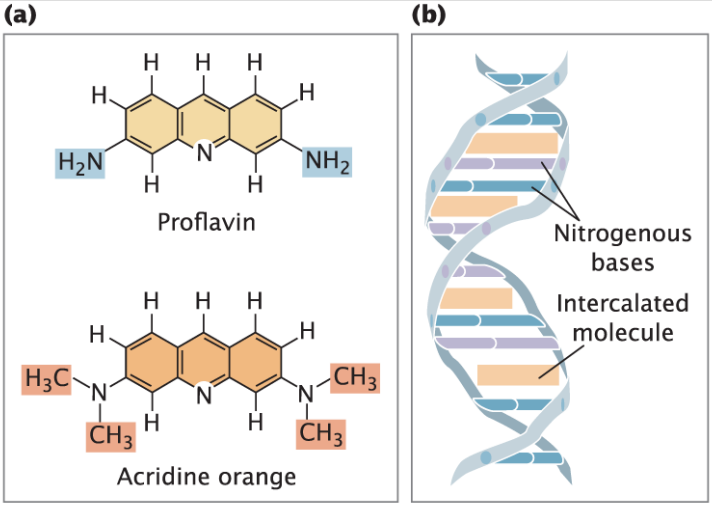
18.20 Oxidative radicals convert guanine into 8-oxy-7,8-dihydrodeoxyguanine.

Description

The illustration shows oxidative radicals convert guanine into 8-oxy-7,8-dihydrodeoxyguanine. The illustration shows guanine on the left. A forward arrow shows text that reads, oxidative radicals. The product, 8-Oxy-7,8-dihydrodeoxyguanine is shown on the right. It has a highlighted oxygen atom which is double bonded to C 8. Text below reads, may mispair with adenine.

INTERCALATING AGENTS

Proflavin, acridine orange, ethidium bromide, and dioxin are **intercalating agents** (Figure 18.21a), which produce mutations by sandwiching themselves (intercalating) between adjacent bases in DNA, distorting the three-dimensional structure of the helix and causing single-nucleotide insertions and deletions in replication (Figure 18.21b). These insertions and deletions frequently produce frameshift mutations, so the mutagenic effects of intercalating agents are often severe. Because intercalating agents generate both insertions and deletions, they can reverse mutations they produce.



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18.21 Intercalating agents are mutagens. Intercalating agents, such as proflavin and acridine orange (a), insert themselves between adjacent bases in DNA, distorting the three-dimensional structure of the DNA double helix (b).

Description

Part a on the left shows a proflavin at the top. An amino group is present at its C 3 and another amino group is present at C 6. Both the amino groups are highlighted. An Acridine orange is present below. The structure of Acridine orange is similar to that of proflavin except the N atoms at C 3 and C 6, have two methyl groups each instead of H atoms. All four methyl groups are highlighted. Part b on the right shows a double helical D N A strand with nitrogenous bases at the center. There is a thick Intercalated molecule after every two bases. The intercalated molecules distort the shape of the D N A double helix.

CONCEPTS

Mutagenic chemicals can produce mutations by a number of mechanisms. Base analogs are incorporated into DNA and frequently pair with the wrong base. Alkylating agents, deaminating chemicals, hydroxylamine, and oxidative radicals change the structure of DNA bases, thereby altering their pairing properties. Intercalating agents wedge between the bases and cause single-base insertions and deletions in replication.

✔ CONCEPT CHECK 4

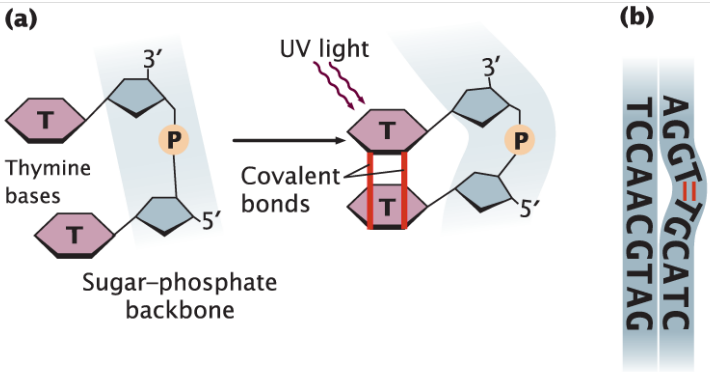
Base analogs are mutagenic because of which characteristic?

a. They produce changes in DNA polymerase that cause it to malfunction.
b. They distort the structure of DNA.
c. They are similar in structure to the normal bases.
d. They chemically modify the normal bases.

Radiation

In 1927, Hermann Muller demonstrated that mutations in fruit flies could be induced by X-rays. The results of subsequent studies showed that X-rays greatly increase mutation rates in all organisms. Because of their high energies, X-rays, gamma rays, and cosmic rays are all capable of penetrating tissues and damaging DNA. These forms of radiation, called ionizing radiation, dislodge electrons from the atoms they encounter, changing stable molecules into free radicals and reactive ions, which then alter the structures of bases and break phosphodiester bonds in DNA. Ionizing radiation also frequently results in double-strand breaks in DNA. Attempts to repair these breaks can produce chromosome mutations (discussed in Chapter 8).

Ultraviolet (UV) light has less energy than ionizing radiation and does not dislodge electrons but is nevertheless highly mutagenic. Pyrimidine bases readily absorb UV light, which causes chemical bonds to form between adjacent pyrimidine molecules on the same strand of DNA, creating **pyrimidine dimers** (**Figure 18.22a**). Pyrimidine dimers consisting of two thymine bases (called thymine dimers) are most frequent, but cytosine dimers and thymine–cytosine dimers can also form. These dimers are bulky lesions that distort the configuration of DNA (**Figure 18.22b**) and often block replication. Most pyrimidine dimers are immediately repaired by mechanisms discussed in Section 18.5, but some escape repair and inhibit replication and transcription.



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18.22 Pyrimidine dimers result from ultraviolet light. (a) Formation of a thymine dimer. (b) A thymine dimer distorts the DNA molecule.

Description

Part a on the left shows a sugar–phosphate backbone running from 3 prime at the top to 5 prime at the bottom. The two pentose sugars have thymine bases (T) on the C 1 and a phosphate molecule is present between C 4 of both the sugars. A forward arrow labeled covalent bonds is present. When the sugar phosphate backbone is exposed to U V light, two covalent bonds are also formed between the thymine bases. The covalent bonds are shown by thick red vertical lines between two corresponding carbon atoms of the thymine bases. C 2 of first thymine base is covalently bonded to C 2 of the second thymine base. C 3 of the first thymine base is covalently bonded to C 3 of second thymine base.

Part b on the right shows a thymine dimer. The first thymine molecule has the following sequence from top to bottom: T, C, C, A, A, C, G, T, A, G. The second thymine molecule has the corresponding sequence: A, G, G, T double bonded to T, G, C, A, T, C. The double bond is represented by red, vertical parallel lines.

When pyrimidine dimers block replication, cell division is inhibited and the cell usually dies; for this reason, UV light kills bacteria and is an effective sterilizing agent. For a mutation—a hereditary error in the genetic instructions—to occur, the replication block must be overcome. Bacteria can sometimes circumvent replication blocks

produced by pyrimidine dimers and other types of DNA damage by means of the **SOS system**. This system can overcome these blocks and allow replication to proceed, but in the process, it makes numerous mistakes and greatly increases the rate of mutation. Indeed, the very reason that replication can proceed in the presence of a block is that the enzymes in the SOS system do not strictly adhere to the base-pairing rules. The trade-off is that replication continues and the cell survives, but only by sacrificing the normal accuracy of DNA synthesis.

CONCEPTS

Ionizing radiation such as X-rays and gamma rays damages DNA by dislodging electrons from atoms; these electrons then break phosphodiester bonds and alter the structure of bases. Ultraviolet light causes mutations primarily by producing pyrimidine dimers that disrupt replication and transcription. The SOS system enables bacteria to overcome replication blocks but introduces mistakes in replication.

18.3 Mutations Are the Focus of Intense Study by Geneticists

Because mutations often have detrimental effects, they are frequently studied by geneticists. These studies have included the development of tests to determine the mutagenic properties of chemical compounds and the investigation of human populations tragically exposed to high levels of radiation.

Detecting Mutagens with the Ames Test

People in industrial societies are surrounded by a multitude of artificially produced chemicals: more than 50,000 different chemicals are in commercial and industrial use today, and from 500 to 1000 new chemicals are introduced each year. Some of these chemicals are potential **carcinogens** (substances capable of causing cancer). Many natural products are also potentially carcinogenic. One method for testing the cancer-causing potential of substances is to administer them to laboratory animals (rats or mice) and compare the incidence of cancer in the treated animals with that in the control animals. Unfortunately, these tests are time-consuming and expensive. Furthermore, the ability of a substance to cause cancer in

rodents is not always indicative of its effect on humans. After all, we aren't rats!

In 1974, Bruce Ames developed a simple test for evaluating the potential of chemicals to cause cancer. The **Ames test** is based on the principle that both cancer and mutations result from damage to DNA, and the results of experiments have demonstrated that 90% of known carcinogens are also mutagens. Ames proposed that mutagenesis in bacteria could serve as an indicator of carcinogenesis in humans.

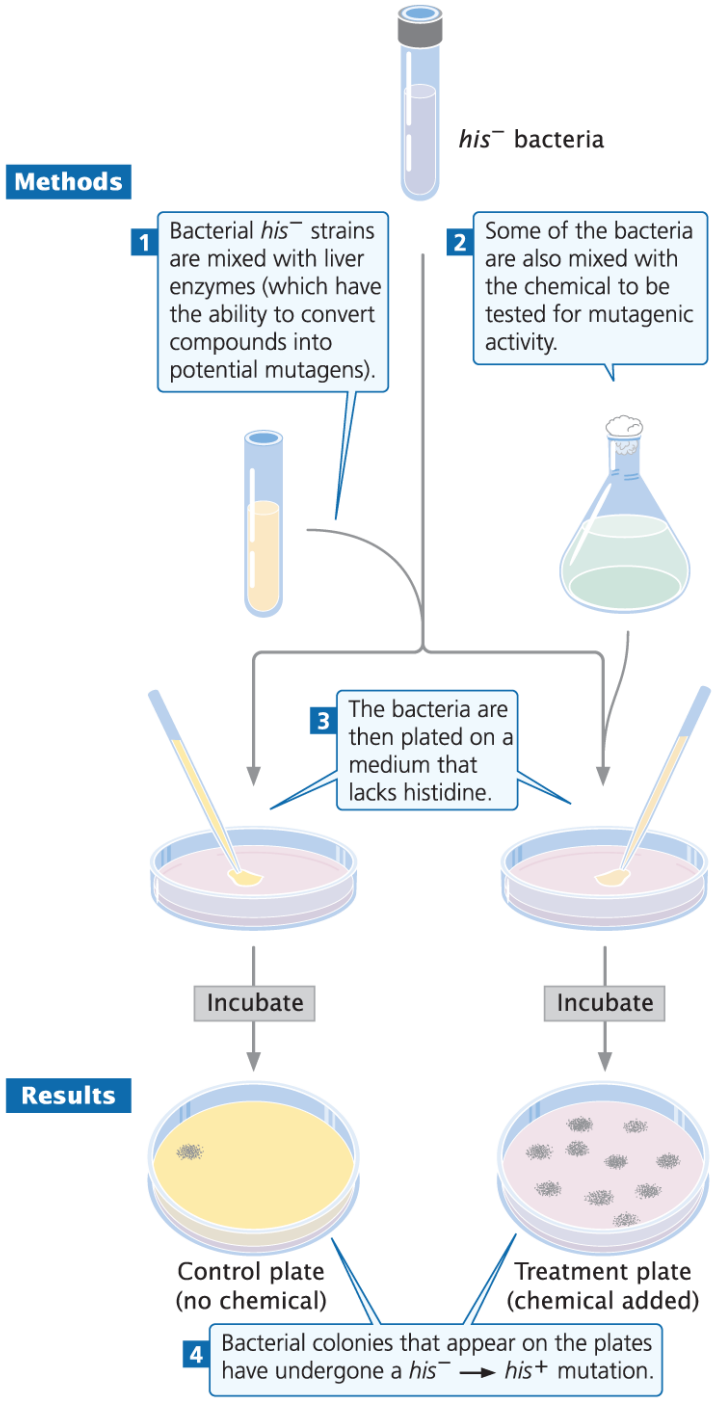
The Ames test uses auxotrophic strains of the bacterium *Salmonella typhimurium* with traits that make them particularly susceptible to mutagens. These strains have defects in the lipopolysaccharide coat, which normally protects the bacteria from chemicals in the environment, and their DNA-repair systems have been inactivated. A recent version of the test (called Ames II) uses several auxotrophic strains that detect different types of base substitutions. Other strains detect different types of frameshift mutations.

Some compounds are not active carcinogens but can be converted into cancer-causing compounds in the body. To make the Ames test sensitive to such *potential* carcinogens, a compound to be tested is first incubated in mammalian liver extract that contains metabolic enzymes.

Each strain of bacteria used in the test carries a *his*$^{-}$ mutation, which renders it unable to synthesize the amino acid histidine. When the bacteria are plated onto a medium that lacks histidine (**Figure 18.23**), only bacteria that have undergone a reverse mutation of the histidine gene (*his*$^{-} \rightarrow$ *his*$^{+}$) are able to synthesize histidine and grow on the medium, which makes these mutations easy to detect. Different dilutions of a chemical to be tested are added to the bacteria and plates inoculated with the bacteria. The number of mutated bacterial colonies that appear on each plate is compared with the number that appear on control plates with no chemical (i.e., that arose through spontaneous mutation). Any chemical that significantly increases the number of colonies appearing on a treated plate is mutagenic and probably also carcinogenic.

Experiment

Question: How can chemicals be quickly screened for their ability to cause cancer?



Conclusion: Any chemical that significantly increases the number of colonies appearing on the treatment plate is mutagenic and therefore probably also carcinogenic.

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18.23 The Ames test is used to identify chemical mutagens.

Description

The illustration shows a question at the top that reads, How can chemicals be quickly screened for their ability to cause cancer? A test tube with a liquid is labeled small H small I small S superscript negative bacteria. Methods section is present below. A downward arrow from the test tube divides into two arrows below. The left side shows a test tube filled with liver enzymes. A caption reads, Bacterial small H small I small S superscript negative strains are mixed with liver enzymes (which have the ability to convert compounds into potential mutagens). A conical flask on the right, closed with a cotton plug, is filled with a chemical. A caption reads, Some of the bacteria are also mixed with the chemical to be tested for mutagenic activity. The arrow on the left shows a petri dish with a medium that lacks histidine. A dropper filled with the small H small I small S superscript negative strains that were mixed with liver enzymes is used to plate the bacteria. The arrow on the right shows a petri dish with a medium that lacks histidine. A dropper filled with the small H small I small S superscript negative strains that were mixed with chemical is used to plate the bacteria. A caption reads, The bacteria are then plated on a medium that lacks histidine. Both the petri dishes are incubated. The results section below shows that the petri dish on the left labeled control plate (no chemical) has only one colony. The petri dish becomes the same color as of the bacteria mixture with liver enzymes, in this case yellow. The petri dish on the right labeled treatment plate (chemical added) has only multiple colonies. A caption reads, Bacterial colonies that appear on the plates have undergone a small H small I small S superscript negative to small H small I small S superscript positive mutation. A caption below reads, Conclusion: Any chemical that significantly increases the number of colonies appearing on the treatment plate is mutagenic and therefore probably also carcinogenic.

The Ames test has been applied to thousands of chemicals and commercial products. An early demonstration of its usefulness was the discovery in 1975 that many hair dyes sold in the United States at that time contained compounds that were mutagenic to bacteria. These compounds were then removed from most hair dyes.

CONCEPTS

The Ames test uses *his*$^-$ strains of bacteria to test chemicals for their ability to produce *his*$^-$ → *his*$^+$ mutations. Because mutagenic activity and carcinogenic potential are closely correlated, the Ames test is widely used to screen chemicals for their cancer-causing potential.

Effects of Radiation Exposure in Humans

People are routinely exposed to low levels of radiation from cosmic, medical, and environmental sources, but there have also been tragic events that produced exposures of much higher degree.

On August 6, 1945, toward the end of World War II, a high-flying American airplane dropped a single atomic bomb on the city of Hiroshima, Japan. The explosion devastated an area of the city measuring 4.5 square miles, killed between 90,000 and 140,000 people, and injured almost as many (**Figure 18.24**). Three days later, the United States dropped another atomic bomb on the city of Nagasaki, this time destroying an area measuring 1.5 square miles and killing between 60,000 and 80,000 people. Huge amounts of radiation were released during these explosions, and many people were exposed.

Bettmann/Getty Images.

18.24 Hiroshima was destroyed by an atomic bomb on August 6, 1945. The atomic explosion produced many somatic mutations among the survivors.

After the war, a joint Japanese–U.S. effort was made to study the biological effects of radiation exposure on the survivors of the atomic blasts and their children. Somatic mutations were assessed by studying radiation sickness and cancer among the survivors; germ-line mutations were assessed by looking at birth defects, chromosome abnormalities, and gene mutations in children born to people who had been exposed to radiation.

Geneticist James Neel and his colleagues examined almost 19,000 children of people who were within 2000 meters (1.2 miles) of the center of the atomic blast at Hiroshima or Nagasaki, along with a similar number of children whose parents did not receive radiation exposure. Radiation doses were estimated for a child's parents on the basis of careful assessment of the parents' location, posture, and

position at the time of the blast. A blood sample was collected from each child, and gel electrophoresis was used to investigate amino acid substitutions in 28 proteins. When rare variants were detected, blood samples from the child's parents were also analyzed to establish whether the variant was an inherited or a new mutation.

Of a total of 289,868 genes examined by Neel and his colleagues, only one mutation was found in the children of exposed parents; no gene mutations were found in the control group. From these findings, a mutation rate of 3.4×10^{-6} was estimated for the children whose parents were exposed to the blasts, which is within the range of spontaneous mutation rates observed for other eukaryotes. Neel and his colleagues also examined the frequency of chromosome mutations, the frequency of aneuploidy, and the sex ratios of children born to exposed parents. There was no evidence in any of these assays for increased mutations among the children of the people who were exposed to radiation from the atomic explosions, suggesting that germ-line mutations were not elevated.

Animal studies clearly show that radiation causes germ-line mutations, so why was there no apparent increase in germ-line mutations among the inhabitants of Hiroshima and Nagasaki? The exposed parents did exhibit an increased incidence of leukemia and other types of cancers, so somatic mutations were clearly induced. The answer to the question is not known, but the lack of germ-line mutations may be due to the fact that those people who received the largest radiation doses died soon after the blasts. Additional insights

into the genetic effects of radiation have come from studies of people exposed to radiation in the Chernobyl nuclear accident in 1986 and other nuclear accidents, as well as to radiation used in medicine and industry.

THINK-PAIR-SHARE Question 5

18.4 Transposable Elements Can Cause Mutations

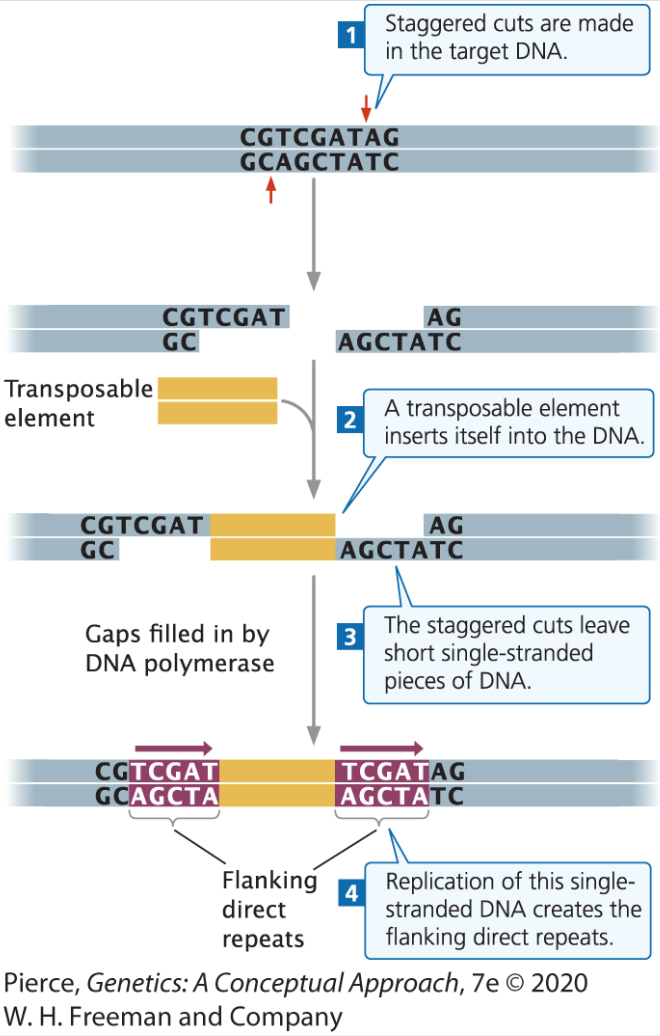
Transposable elements—DNA sequences that can move about in the genome—are often a cause of mutations. These mobile DNA elements have been given a variety of names, including transposons, transposable genetic elements, movable genes, controlling elements, and jumping genes. They are found in the genomes of all organisms and are abundant in many, including our own. Most transposable elements can insert themselves at many different locations in the genome, relying on mechanisms that are distinct from homologous recombination. Through their movement (transposition), transposable elements often cause mutations, either by inserting into a gene and disrupting it or by promoting chromosome rearrangements, such as deletions, duplications, and inversions (see Chapter 8).

General Characteristics of Transposable Elements

There are many different types of transposable elements: some have simple structures, encompassing only those sequences necessary for their own transposition, whereas others have complex structures and encode a number of functions not directly related to

transposition. Despite this variation, many transposable elements have certain features in common.

Short **flanking direct repeats**—short, directly repeated sequences from 3 to 12 bp long—present on both sides of most transposable elements. The sequences of these repeats vary, but their length is constant for each type of transposable element. These repeats are not a part of the transposable element and do not travel with it. Rather, they are generated in the process of transposition at the point of insertion. Flanking direct repeats are created when staggered cuts are made in the target DNA, as shown in **Figure 18.25**. These staggered cuts leave short single-stranded pieces of DNA on either side of the transposable element. Replication of these single-stranded pieces creates the flanking direct repeats.



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18.25 Flanking direct repeats are generated when a transposable element inserts itself into DNA.

Description

The illustration shows a D N A double strand at the top. The sequence of the top strand is as follows: C, G, T, C, G, A, T, staggered cut, A, G.

The sequence of the bottom strand is as follows: G, C, staggered cut, A, G, C, T, A, T, C. This leads to the fragmentation of the D N A strand into two parts. A caption reads, Staggered cuts are made in the target D N A.

The top strand of first fragment is as follows: C, G, T, C, G, A, T.

The bottom strand of first fragment is as follows: G, C.

The top strand of second fragment is as follows: A, G.

The bottom strand of second fragment is as follows: A, G, C, T, A, T, C.

A double stranded transposable element enters the process. A caption reads, A transposable element inserts itself into the D N A.

The top strand of the transposable element sticks to the right end of the top strand of first fragment below. The bottom strand of the transposable element sticks to the left end of the bottom strand of the second fragment. A caption reads, The staggered cuts leave short single-stranded pieces of D N A.

The gaps after the bottom strand of first fragment and gap prior to the top strand of second fragment, get filled by D N A polymerase.

The final D N A double strand obtained below is as follows:

The sequence of the top strand is as follows: C, G, flanking direct repeat starts T, C, G, A, T, transposable element, T, C, G, A, T flanking direct repeat ends, A, G.

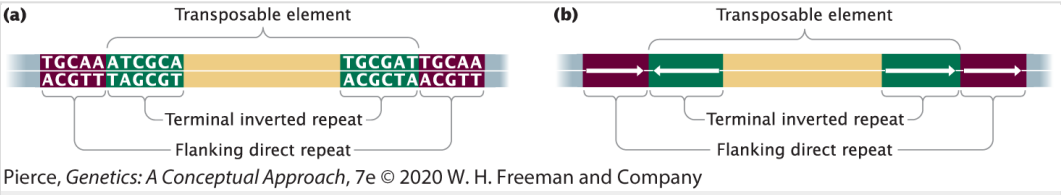
The sequence of the bottom strand is as follows: G, C, flanking direct repeat starts A, G, C, T, A, transposable element, A, G, C, T, A, flanking direct repeat ends, T, C. A forward arrow appears over the flanking direct repeats on the top strand. A caption reads, Replication of this single stranded D N A creates the flanking direct repeats.

At the ends of many, but not all, transposable elements are **terminal inverted repeats**—sequences from 9 to 40 bp in length that are inverted complements of each other. For example, the following sequences are inverted repeats:

$$5'-\text{ACAGTTCAG} \ldots \text{CTGAACTGT}-3'$$
$$3'-\text{TGTCAAGTC} \ldots \text{GACTTGACA}-5'$$

On the same strand, the two sequences are not simple inversions, as their name might imply; rather, they are both inverted and

complementary. (Notice that the sequence from left to right in the top strand is the same as the sequence from right to left in the bottom strand.) Terminal inverted repeats are recognized by enzymes that catalyze transposition, and they are required for transposition to take place. Figure 18.26 summarizes the general characteristics of transposable elements. ❯ **TRY** PROBLEM 34



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18.26 Many transposable elements have common characteristics. (a) Most transposable elements generate flanking direct repeats on each side of the point of insertion into target DNA. Many transposable elements also possess terminal inverted repeats. (b) These representations of direct and inverted repeats are used in illustrations throughout this chapter.

Description

Part a on the left shows a D N A double strand.

The sequence of the top strand is as follows: Flanking direct repeat starts T, G, C, A, A Flanking direct repeat ends, transposable element starts terminal inverted repeat starts A, T, C, G, C, A terminal inverted repeat ends terminal inverted repeat starts T, G, C, G, A, T terminal inverted repeat ends Flanking direct repeat starts T, G, C, A, A Flanking direct repeat ends.

The sequence of the bottom strand is as follows: Flanking direct repeat starts A, C, G, T, T Flanking direct repeat ends, transposable element starts terminal inverted repeat starts T, A, G, C, G, T terminal inverted repeat ends terminal inverted repeat starts A, C, G, C, T, A terminal inverted repeat ends Flanking direct repeat starts A, C, G, T, T Flanking direct repeat ends.

Part b on the right shows a D N A double strand.

Flanking direct repeat represented by forward arrow, transposable element starts, terminal inverted repeat represented by backward arrow, terminal inverted repeat represented by forward arrow, transposable element ends, Flanking direct repeat represented by forward arrow.

CONCEPTS

Transposable elements are mobile DNA sequences that often cause mutations. There are many different types of transposable elements; most generate short flanking direct repeats at the target sites as they insert. Many transposable elements also possess short terminal inverted repeats.

✔ CONCEPT CHECK 5

How are flanking direct repeats created in transposition?

The Process of Transposition

As mentioned previously, **transposition** is the movement of a transposable element from one location to another. Several different mechanisms are used for transposition in both prokaryotic and eukaryotic cells. Nevertheless, all types of transposition share several steps: (1) staggered breaks are made in the target DNA (see Figure 18.25); (2) the transposable element is joined to single-stranded ends of the target DNA; and (3) DNA is replicated at the single-strand gaps. A **transposase** enzyme, often encoded by the

transposable element, is used to make the staggered breaks in DNA and to integrate the transposable element into a new site.

Some transposable elements transpose as DNA and are referred to as **DNA transposons** (or *Class II transposable elements*). Other transposable elements, called **retrotransposons** or *Class I transposons*, transpose through an RNA intermediate. In this case, RNA is transcribed from the transposable element (DNA) and is then copied back into DNA by a special enzyme called reverse transcriptase. Transposable elements found in bacteria are DNA transposons. Both DNA transposons and retrotransposons are found in eukaryotes, although retrotransposons are more common.

Among DNA transposons, transposition may be replicative or nonreplicative. In **replicative transposition** (also called *copy-and-paste transposition*), a new copy of the transposable element is introduced at a new site while the old copy remains behind at the original site, so the number of transposable element copies increases as a result of transposition. In **nonreplicative transposition** (*cut-and-paste transposition*), the transposable element is excised from the old site and inserted at a new site without any increase in the number of its copies. Nonreplicative transposition requires the replication of only the few nucleotides that constitute the flanking direct repeats. Retrotransposons use replicative transposition only.

CONTROL OF TRANSPOSITION

Many organisms limit transposition by methylating the DNA in regions where transposons are common. DNA methylation usually suppresses transcription (see Chapter 17), preventing the production of the transposase enzyme necessary for transposition. Alterations of chromatin structure are also used to prevent the transcription of transposons. In other cases, translation of the transposase mRNA is controlled. Some animals use small RNA molecules called Piwi-interacting RNAs (piRNAs, see Chapter 14) to silence transposons; piRNAs combine with Piwi proteins and inhibit the expression of transposon sequences.

TRANSPOSITION IN HUMANS

About 45% of the human genome comprises sequences that are related to transposable elements, mostly retrotransposons (some research suggests that almost two-thirds of the human genome consists of transposable elements). Researchers previously assumed that most of these transposable elements are inactive and that little transposition occurs today, although it clearly took place extensively during our past evolution. More recently, however, researchers have begun to map copies of transposons across the human genome and have discovered that people often differ in the numbers and locations of their transposons. This finding suggests that recent transpositions are more common than previously thought. The L1

transposon, for example, is estimated to undergo one transposition event in about every 100 human births.

Research has also demonstrated that some cancer cells have elevated levels of transposition, probably because patterns of DNA methylation that normally inhibit transposition are disrupted in these cells.

CONCEPTS

Transposition may take place through DNA or through an RNA intermediate. In replicative transposition, a new copy of the transposable element inserts in a new location and the old copy stays behind; in nonreplicative transposition, the old copy excises from the old site and moves to a new site. Transposition through an RNA intermediate requires reverse transcription to integrate into the target site. Many cells regulate transposition through a variety of mechanisms.

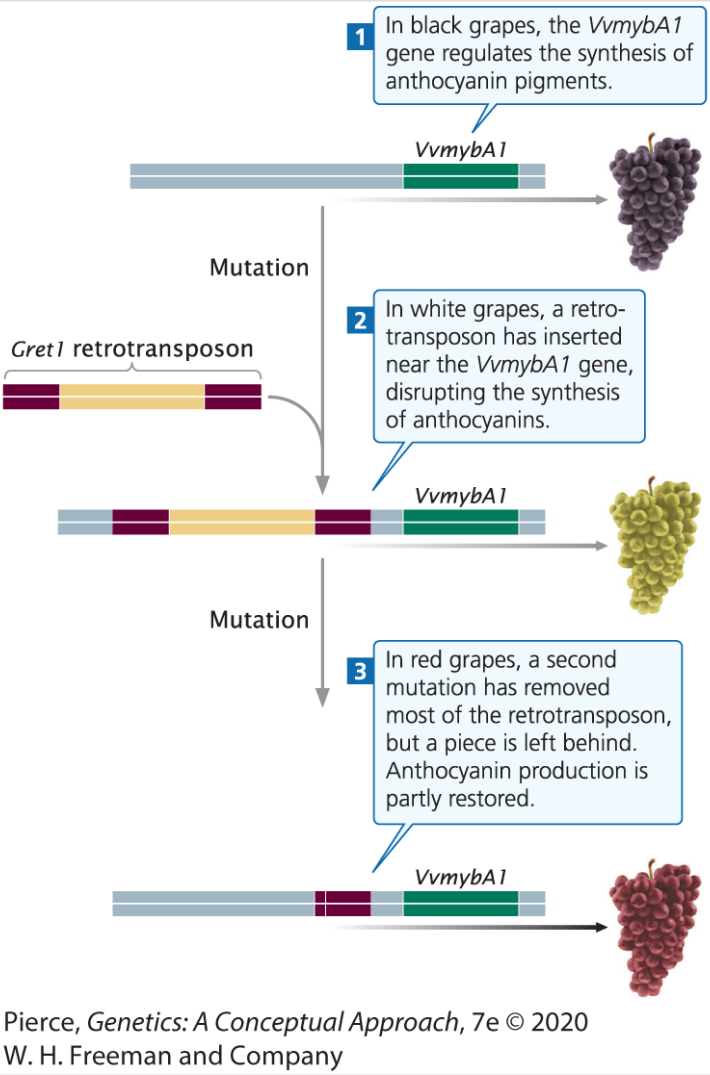
The Mutagenic Effects of Transposition

Because transposable elements can insert into genes and disrupt their function, transposition is generally mutagenic. In fact, more than half of all spontaneously occurring mutations in *Drosophila* result from the insertion of a transposable element in or near a functional gene.

A number of cases of human genetic disease have been traced to the insertion of a transposable element into a vital gene. For example, insertion of the L1 transposable element into the gene for blood-clotting factor VIII has caused hemophilia. Although most mutations resulting from transposition are detrimental, transposition may occasionally activate a gene or change the phenotype of the cell in a beneficial way. For instance, bacterial transposable elements sometimes carry genes that encode antibiotic resistance, and several transposable elements have created mutations that confer insecticide resistance in insects.

A dramatic example of the mutagenic effect of transposable elements is seen in the color of grapes, which come in black, red, and white varieties (**Figure 18.27**). Black and red grapes result from the production of red pigments, called anthocyanins, in the skin, which are lacking in white grapes. White grapes resulted from a mutation in black grapes that turned off the production of anthocyanins. This mutation consisted of the insertion of a 10,422-bp retrotransposon called *Gret1* near a gene that promotes the production of anthocyanins. The *Gret1* retrotransposon apparently disrupted sequences that regulate the gene, effectively shutting down anthocyanin production and producing a white grape with no anthocyanins. Interestingly, red grapes resulted from a second mutation that occurred in white grapes (see Figure 18.27). This mutation (probably resulting from faulty recombination) removed most, but not all, of the retrotransposon, switching anthocyanin

production back on, but not as intensely as in the original black grapes.



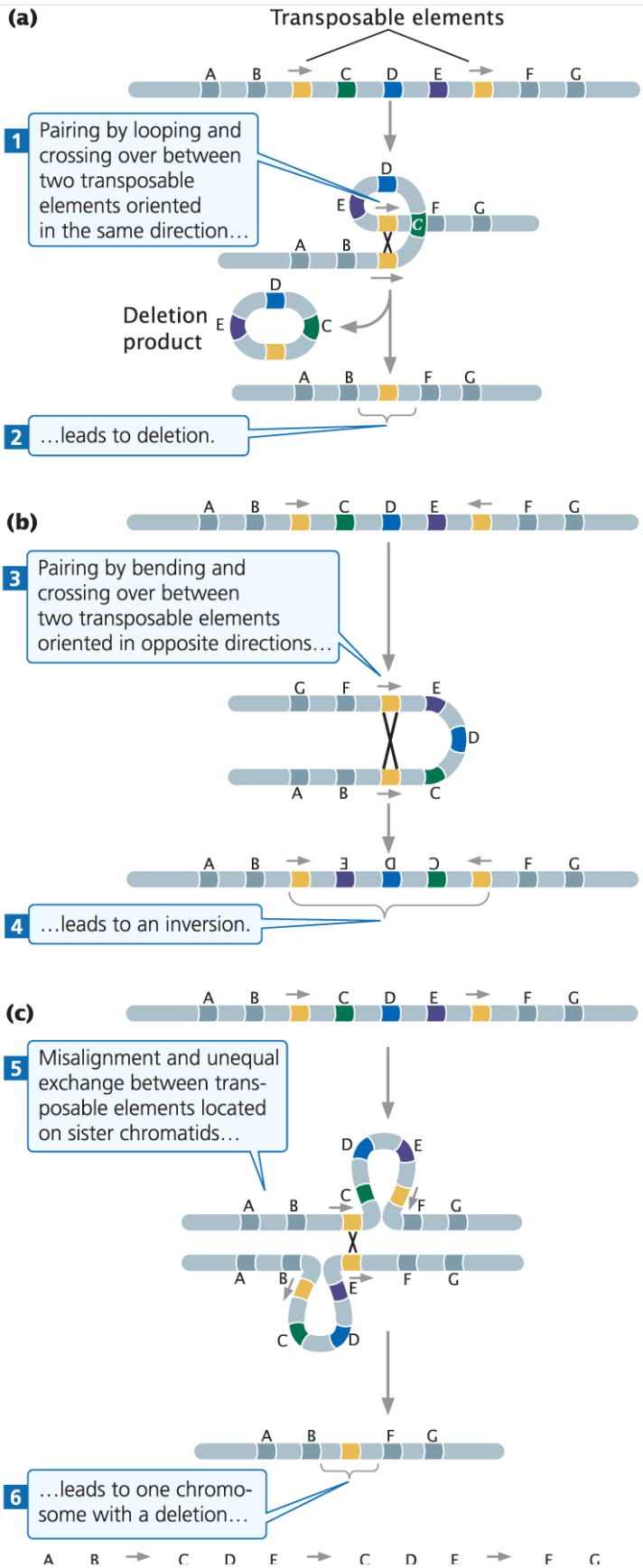
18.27 White and red color in grapes resulted from insertion and deletion of a retrotransposon, respectively.

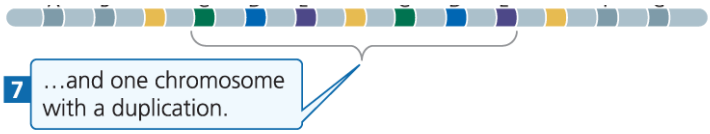
Description

The illustration shows a D N A double strand with a gene Big V small V small M small Y small B big A 1, at the top. A caption reads, In black grapes, the Big V small V small M small Y small B big A 1 gene regulates the synthesis of anthocyanin pigments. A bunch of black grapes is present on the right. A downward arrow shows mutation. A G r e t 1

retrotransposon enters the process and inserts in the D N A double strand near the Big V small V small M small Y small B big A 1 gene below. A caption reads, In white grapes, a retrotransposon has inserted near the Big V small V small M small Y small B big A 1 gene, disrupting the synthesis of anthocyanins. A bunch of white grapes is present on the right. A downward arrow shows mutation and transposon is deleted and only a part of it remains. The gene Big V small V small M small Y small B big A 1 remains intact. A bunch of red grapes is present on the right. A caption reads, In red grapes, a second mutation has removed most of the retrotransposon, but a piece is left behind. Anthocyanin production is partly restored.

Because transposition entails the exchange of DNA sequences and recombination, it often leads to DNA rearrangements. Homologous recombination between multiple copies of transposons can lead to duplications, deletions, and inversions, as shown in **Figure 18.28**. The *Bar* mutation in *Drosophila* (see Figure 8.7) is a tandem duplication thought to have arisen through homologous recombination between two copies of a transposable element present in different locations on the X chromosome. Similarly, recombination between copies of the transposable element *Rider* caused a duplication that results in elongate fruit in tomatoes.


(a)
Transposable elements
A B C D E F G
1 Pairing by looping and crossing over between two transposable elements oriented in the same direction…
Deletion product
2 …leads to deletion.
(b)
3 Pairing by bending and crossing over between two transposable elements oriented in opposite directions…
4 …leads to an inversion.
(c)
5 Misalignment and unequal exchange between transposable elements located on sister chromatids…
6 …leads to one chromosome with a deletion…



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18.28 Many chromosomal rearrangements are generated by transposition.

Description

Part a at the top shows a rod shaped chromosome with the following gene sequence: Gene big A, Gene big B, Transposable element with a forward arrow over it, Gene big C, Gene big D, Gene big E, Transposable element with a forward arrow over it, Gene big F, Gene big G. The two transposable elements cross over by forming a loop with genes big C, big D, big E. The genes big C, big D, big E along with a transposable element form a circular structure and are eliminated. This structure is labeled deletion product. The obtained chromosome below has the following sequence: Gene big A, Gene big B, Transposable element, Gene big F, Gene big G. A caption reads, Pairing by looping and crossing over between two transposable elements oriented in the same direction leads to deletion.

Part b below shows a rod shaped chromosome with the following gene sequence: Gene big A, Gene big B, Transposable element with a forward arrow over it, Gene big C, Gene big D, Gene big E, Transposable element with a backward arrow over it, Gene big F, Gene big G. The chromosome forms a bend at the right side so that the transposable elements cross over. The resultant chromosome obtained below has the following sequence: Gene big A, Gene big B, Transposable element with a forward arrow over it, Gene big E, Gene big D, Gene big C, Transposable element with a backward arrow over it, Gene big F, Gene big G. A caption reads, Pairing by bending and crossing over between two transposable elements oriented in opposite directions leads to inversion.

Part c below shows a rod shaped chromosome with the following gene sequence: Gene big A, Gene big B, Transposable element with a forward arrow over it, Gene big C, Gene big D, Gene big E, Transposable element with a forward arrow over it, Gene big F, Gene big G. The chromatid loops out below and has gene big C, big D, big E, and big F in the loop. A sister chromatid below it also loops out with a transposable element, big C, big D, and big E. Other remaining genes on both the chromatids are aligned with gene big A under big A, big B under big B, big F under big F, and big G under big G. The first

transposable element on the first chromatid crosses over with second transposable element on the sister chromatid below. The first resultant chromosome obtained below has the following sequence: Gene big A, Gene big B, Transposable element, Gene big F, Gene big G. The second resultant chromosome obtained below has the following sequence: Gene big A, Gene big B, Transposable element with a forward arrow over it, Gene big C, Gene big D, Gene big E, Transposable element with a forward arrow over it, Gene big C, Gene big D, Gene big E, Transposable element with a forward arrow over it, Gene big F, Gene big G. A caption reads, Misalignment and unequal exchange between transposable elements located on sister chromatids leads to one chromosome with a deletion and one chromosome with a duplication.

DNA rearrangements can also be caused by the excision of transposable elements in nonreplicative transposition. If the broken DNA is not repaired properly, a DNA rearrangement can be generated.

Because most transposable elements insert randomly into DNA sequences, they provide researchers with a powerful tool for inducing mutations throughout the genome, allowing them to determine the functions of genes, study genetic phenomena, and map genes. Furthermore, because the transposable element being used has a known sequence, it can serve as a "tag" for locating a gene in which a mutation has occurred. For example, researchers engineered a transposable element named *Sleeping Beauty* to induce mutations in mice and used it to search for genes that cause cancer. *Sleeping Beauty* was introduced into a strain of mice that produce the transposase needed for transposition. The transposable element inserted randomly into different locations in the genome; occasionally, it inserted into a gene that protects against cancer and

destroyed its function. By looking for the *Sleeping Beauty* sequence in the DNA from the tumor cells that subsequently developed, geneticists identified a number of genes that protect against cancer.

Bacteria and eukaryotic organisms possess a number of different types of transposable elements, the structures of which vary extensively. Next, we consider the structures and types of transposable elements in bacteria and eukaryotes.

CONCEPTS

Transposable elements frequently cause mutations and DNA rearrangements.

✔ CONCEPT CHECK 6

Briefly explain how transposition causes mutations and chromosome rearrangements.

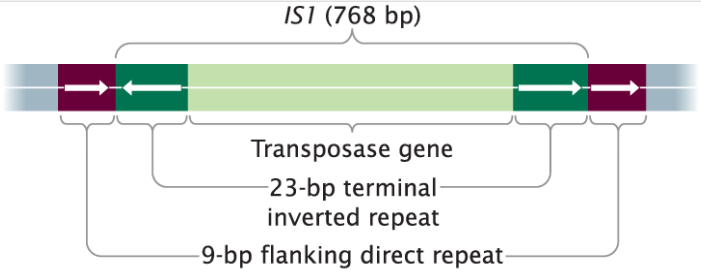
Transposable Elements in Bacteria

The DNA transposons found in bacteria constitute two major groups: (1) simple transposable elements, called insertion sequences, that carry only the information required for movement; and (2) more complex transposable elements that contain DNA sequences not directly related to transposition.

INSERTION SEQUENCES

The simplest type of transposable element in bacterial chromosomes and plasmids is an **insertion sequence (IS)**. This type of element carries only the genetic information necessary for its movement. Insertion sequences are common constituents of bacteria; they can also infect plasmids and viruses and, in this way, can be passed from one cell to another. Geneticists designate each type of insertion sequence with *IS* followed by an identifying number. For example, *IS1* is a common insertion sequence found in *E. coli*.

A number of different insertion sequences have been found in bacteria. They are typically from 800 to 2000 bp in length and possess the two hallmarks of transposable elements: terminal inverted repeats and the generation of flanking direct repeats at the site of insertion. Most insertion sequences contain one or two genes that encode transposase. *IS1*, a typical insertion sequence, is shown in Figure 18.29. ❯ **TRY** PROBLEM 37



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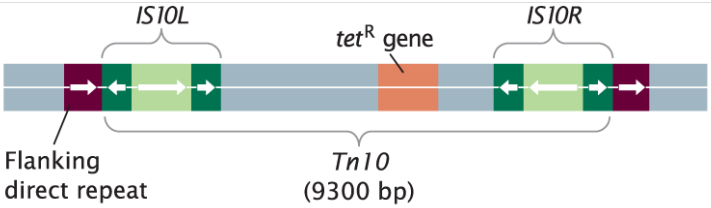
18.29 Insertion sequences such as *IS1* are simple transposable elements found in bacteria.

Description
The illustration shows a double stranded D N A. A 9 b p flanking direct repeat is present on the extreme left and extreme right end of the D N A. It is represented by a forward arrow. A 23 b p terminal inverted repeat is present to the right of the left flanking direct repeat and is represented by a backward arrow. It is followed by a transposase gene in the center. A 23 b p terminal inverted repeat is present to the right of the transposase gene and is represented by a forward arrow. Both the 23 b p terminal inverted repeats with the transposase gene in the center are collectively labeled I S 1 (768 b p).

COMPOSITE TRANSPOSONS

Any segment of DNA that becomes flanked by two copies of an insertion sequence may itself transpose, in which case it is called a **composite transposon**. Each type of composite transposon is designated by the abbreviation *Tn* followed by a number. The composite transposon *Tn10*, for example, consists of about 9300 bp that carry a gene for tetracycline resistance between two *IS10* insertion sequences (**Figure 18.30**). The two insertion sequences each have terminal inverted repeats, so the composite transposon also ends in inverted repeats. Composite transposons also generate flanking direct repeats at their sites of insertion (see Figure 18.30). The insertion sequences at the ends of a composite transposon may be in the same orientation, or they may be inverted relative to each other (as in *Tn10*).



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18.30 *Tn*10 is a composite transposon found in bacteria.

Description

The illustration shows a double stranded D N A. A flanking direct repeat is present on both the left and right end of the D N A and is represented by a forward arrow. An I S 10 L is present on the right of the left flanking direct repeat. The I S 10 L is divided into three parts: The first part shows a backward arrow, the middle part shows a forward arrow, and the third part shows a forward arrow. A small T small E small T superscript big R gene is present on the right. Another I S 10 L is present on the right. The I S 10 L is divided into three parts: First part shows a backward arrow, middle part shows a backward arrow, and third part shows a forward arrow. Both the I S 10 L along with the small T small E small T superscript big R gene are labeled T n 10 (9300 b p).

The insertion sequences at the ends of a composite transposon are responsible for its transposition. The DNA between the insertion sequences is not required for movement and may carry additional information (such as a gene for antibiotic resistance, as in *Tn10*). Presumably, composite transposons evolve when one insertion sequence transposes to a location close to another of the same type. The transposase produced by one of the insertion sequences then catalyzes the transposition of both, allowing them to move together and carry along the DNA that lies between them. In some composite transposons (such as *Tn10*), one of the insertion sequences may be

defective, so its movement depends on the transposase produced by the other.

NONCOMPOSITE TRANSPOSONS

Some transposable elements in bacteria lack insertion sequences and are referred to as **noncomposite transposons**. Noncomposite transposons possess a gene for transposase and have terminal inverted repeats. For instance, the noncomposite transposon *Tn3* carries genes for transposase and resolvase (an enzyme that functions in recombination), plus a gene that encodes the enzyme β-lactamase, which provides resistance to the antibiotic ampicillin.

CONCEPTS

Insertion sequences are prokaryotic transposable elements that carry only the information needed for transposition. A composite transposon consists of two insertion sequences plus intervening DNA. Noncomposite transposons in bacteria lack insertion sequences but have terminal inverted repeats and carry information not related to transposition. All these transposable elements generate flanking direct repeats at their points of insertion.

✔ CONCEPT CHECK 7

Which type of transposable element possesses terminal inverted repeats?

a. Insertion sequence
b. Composite transposon
c. Noncomposite transposon
d. All of the above

Transposable Elements in Eukaryotes

Eukaryotic transposable elements can be divided into two groups. One group is structurally similar to transposable elements in bacteria, typically ending in short inverted repeats and transposing as DNA; examples include the *P* elements in *Drosophila* and the *Ac* and *Ds* elements in corn (maize). The other group comprises retrotransposons: these elements use RNA intermediates, and many are similar in structure and movement to retroviruses (see Section 9.4). The structure, function, and genomic sequences of some retrotransposons show that they are evolutionarily related to retroviruses. Although their mechanism of movement is fundamentally different from that of other transposable elements, retrotransposons also generate flanking direct repeats at the point of insertion. Retrotransposons include the *Ty* elements in yeast, the *copia* elements in *Drosophila*, and the *Alu* sequences in humans.

Ac AND *Ds* ELEMENTS IN CORN

Transposable elements were first identified in corn more than 50 years ago by Barbara McClintock (**Figure 18.31**). McClintock spent much of her long career studying their properties, and her work stands among the landmark discoveries of genetics. Her results, however, were misunderstood and ignored for many years. Not until molecular techniques were developed in the late 1960s and 1970s was the importance of transposable elements widely accepted. The

significance of McClintock's early discoveries was finally recognized in 1983, when she was awarded the Nobel Prize in physiology or medicine.

Topham/The Image Works.

18.31 Barbara McClintock was the first to discover transposable elements.

McClintock's discovery of transposable elements had its genesis in the early work of Rollins A. Emerson on the corn genes that caused variegated (multicolored) kernels. Most corn kernels are either wholly pigmented or colorless (yellow), but Emerson noted that some yellow kernels had spots or streaks of color (Figure 18.32). He proposed that these kernels resulted from an unstable mutation: a mutation in the wild-type gene for pigment produced a colorless kernel, but in some cells, the mutation reverted to the wild type, causing a spot of pigment. However, Emerson didn't know why this mutation was unstable.

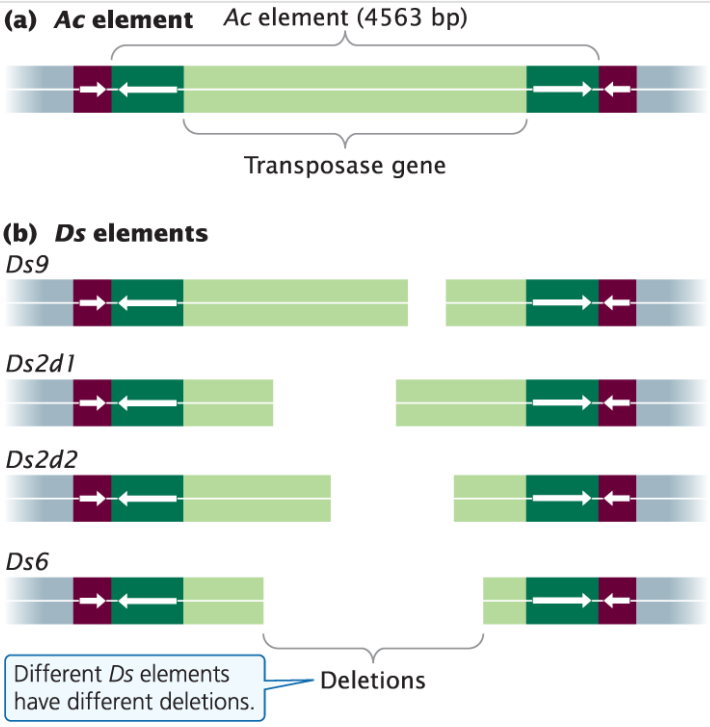
Matt Meadows/Getty Images.

18.32 Variegated (multicolored) kernels in corn are caused by mobile genes. The study of variegated corn kernels led Barbara McClintock to discover transposable elements.

McClintock discovered that the cause of the unstable mutation was a gene that moved. She noticed that chromosome breakage in corn often occurred at a gene that she called *Dissociation* (*Ds*), but only if another gene, the *Activator* (*Ac*), was also present. Occasionally, the genes moved together to a different chromosomal location. McClintock called these moving genes controlling elements because they controlled the expression of other genes.

Since the significance of McClintock's work was recognized, *Ac* and *Ds* elements in corn have been examined in detail. They are DNA transposons that possess terminal inverted repeats and generate flanking direct repeats at the points of insertion. Each *Ac* element contains a single gene that encodes a transposase enzyme (**Figure 18.33a**). Thus, *Ac* elements are *autonomous*—able to transpose. *Ds* elements are *Ac* elements containing one or more deletions that

have inactivated the transposase gene (Figure 18.33b). Unable to transpose on their own (*nonautonomous*), *Ds* elements can transpose in the presence of *Ac* elements because they still possess terminal inverted repeats recognized by Ac transposase.



18.33 *Ac* and *Ds* are transposable elements in corn.

Description

Part a at the top shows A c element. A double stranded D N A shows the following sequence: flanking direct repeat represented by a forward arrow, terminal inverted repeat represented by a backward arrow, transposase gene, terminal inverted repeat represented by a forward arrow, flanking direct repeat represented by a backward arrow. Both the terminal inverted repeats along with transposase gene is labeled A c element (4563 b p).

Part b below shows 4 different D s elements.

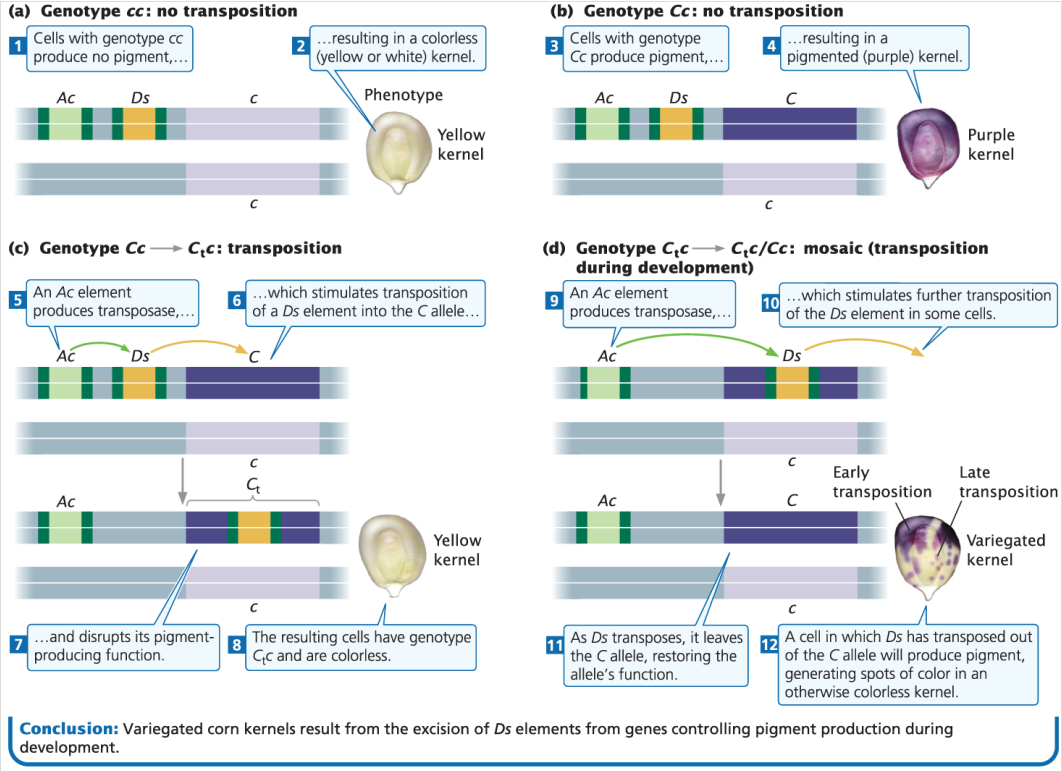
A double stranded D N A representing D s 9 shows the following sequence: flanking direct repeat represented by a forward arrow, terminal inverted repeat represented by a backward arrow, small region of deletion in transposase gene, terminal inverted repeat represented by a forward arrow, flanking direct repeat represented by a backward arrow.

A double stranded D N A representing D s 2 d 1 shows the following sequence: flanking direct repeat represented by a forward arrow, terminal inverted repeat represented by a backward arrow, large region of deletion in transposase gene, terminal inverted repeat represented by a forward arrow, flanking direct repeat represented by a backward arrow.

A double stranded D N A representing D s 2 d 2 shows the following sequence: flanking direct repeat represented by a forward arrow, terminal inverted repeat represented by a backward arrow, large region of deletion in transposase gene, terminal inverted repeat represented by a forward arrow, flanking direct repeat represented by a backward arrow.

A double stranded D N A representing D s 6 shows the following sequence: flanking direct repeat represented by a forward arrow, terminal inverted repeat represented by a backward arrow, very large region of deletion in transposase gene, terminal inverted repeat represented by a forward arrow, flanking direct repeat represented by a backward arrow. A caption reads, Different D s elements have different deletions.

Each kernel in an ear of corn is an individual offspring, originating as an ovule fertilized by a pollen grain. A kernel's pigment pattern is determined by several loci. A pigment-encoding allele at one of these loci can be designated *C*, and an allele at the same locus that does not produce pigment can be designated *c*. A kernel with genotype *cc* will be colorless—that is, yellow or white (**Figure 18.34a**); a kernel with genotype *CC* or *Cc* will produce pigment and be purple (**Figure 18.34b**).



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18.34 Transposition results in variegated corn kernels.

Description

Part a at the top left shows genotype small C small C: no transposition.

A D N A double strand shows the following sequence: A c, D s, gene small C.

A D N A double strand below shows the following sequence: gene small C. A yellow corn kernel is present to its right with text that reads, phenotype. A caption reads, Cells with genotype small C small C produce no pigment, resulting in a colorless (yellow or white) kernel.

Part b on the top right shows the genotype big C small C: no transposition.

A D N A double strand shows the following sequence: A c, D s, gene big C.

A D N A double strand below shows the following sequence: gene small C. A purple corn kernel is present to its right. A caption reads, Cells with genotype big C small C produce pigment, resulting in a pigmented (purple) kernel.

Part c at the bottom left shows Genotype big C small C to big C subscript small T small C: transposition.

A D N A double strand shows the following sequence: A c, D s, gene big C. A forward arrow from A c points to D s and a forward arrow from D s points to gene big C.

A D N A double strand below shows the following sequence: gene small C.

It leads to the insertion of D s element into gene big C below.

A D N A double strand obtained below shows the following sequence: A c, D s element into gene big C, which is collectively labeled, big C superscript small T. A caption reads, An A c element produces transposase, which stimulates transposition of a D s element into the C allele and disrupts its pigment producing function.

A D N A double strand below shows the following sequence: gene small C. A yellow corn kernel is present at the right with a caption that reads, The resulting cells have genotype big C subscript small T small C and are colorless.

Part d at the bottom right shows Genotype big C subscript small T small C to big C subscript small T small slash big C small C: mosaic (transposition during development).

A D N A double strand shows the following sequence: A c, gene big C subscript small T with D s at the center. A forward arrow from A c points to D s and a forward arrow from D s points beyond the D N A strand. A caption reads, An A c element produces transposase, which stimulates further transposition of the D s element in some cells.

A D N A double strand below shows the following sequence: gene small C.

It leads to the deletion of D s element from gene gene big C subscript small T below.

A D N A double strand obtained below shows the following sequence: A c, gene big C. A caption reads, As D s transposes, it leaves the C allele, restoring the allele’s function.

A D N A double strand below shows the following sequence: gene small C.

A Variegated corn kernel is present at the right. The top part shows purple color and is labeled early transposition and the pale purple dots at the bottom part are labeled, late transposition.

A caption below reads, A cell in which D s has transposed out of the C allele will produce pigment,

generating spots of color in an otherwise colorless kernel.

A caption at the bottom reads, Conclusion: Variegated corn kernels result from the excision of D s elements from genes controlling pigment production during development.

In some kernels, a *Ds* element, transposing under the influence of a nearby *Ac* element, may insert into the *C* allele, destroying its ability to produce pigment (**Figure 18.34c**). An allele inactivated by a transposable element is designated by a subscript t; in this case, the allele would be C_t. If a kernel is initially heterozygous with genotype *Cc*, then after the transposition of *Ds* into the *C* allele, the kernel will have genotype $C_t c$. This kernel will be colorless (white or yellow) because neither the C_t allele nor the *c* allele produces pigment.

As the original one-celled corn embryo develops and divides by mitosis, additional transpositions may take place in some cells. In any cell in which the transposable element excises from the C_t allele and moves to a new location, the *C* allele may be rendered functional again: all cells derived from those in which this event has taken place will have the genotype *Cc* and be purple. The presence of these pigmented cells, surrounded by the colorless ($C_t c$) cells, produces a purple spot or streak (called a sector) in the otherwise yellow kernel (**Figure 18.34d**). The size of the sector varies depending on when the excision of the transposable element from the C_t allele takes place. If excision is early in development, then many cells will contain the

functional *C* allele, and the pigmented sector will be large; if excision is late in development, few cells will have the functional *C* allele, and the pigmented sector will be small. › **TRY PROBLEM 43**

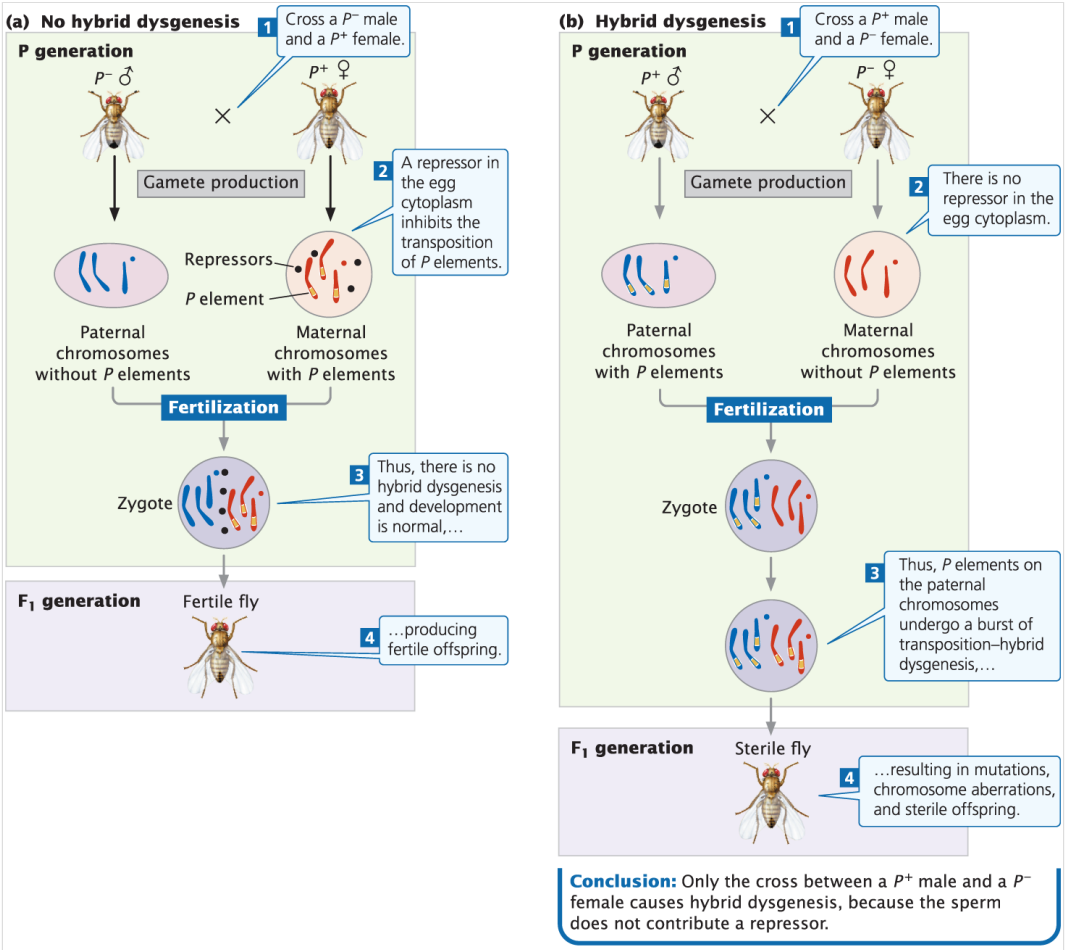
TRANSPOSABLE ELEMENTS IN *DROSOPHILA*

A number of different transposable elements are found in *Drosophila*. One family of *Drosophila* transposable elements comprises the *P* elements. Most functional *P* elements are about 2900 bp long, although shorter *P* elements containing deletions also exist. Each *P* element possesses terminal inverted repeats and generates flanking direct repeats at the site of insertion. Like transposable elements in bacteria, *P* elements are DNA transposons.

Each *P* element encodes both a transposase and a repressor of transposition. The role of the repressor in controlling transposition is demonstrated dramatically in **hybrid dysgenesis**, which is the sudden appearance of numerous mutations, chromosome aberrations, and sterility in the offspring of a cross between a P^+ male fly (*with P* elements) and a P^- female fly (*without* them). The reciprocal cross between a P^+ female and a P^- male produces normal offspring.

Hybrid dysgenesis arises from a burst of transposition when *P* elements are introduced into a cell that does not possess them. In a cell that contains *P* elements, a repressor protein in the cytoplasm

inhibits transposition. When a P^+ female produces eggs, the repressor protein is incorporated into the egg cytoplasm, which prevents further transposition in the embryo and thus prevents mutations from arising. The resulting offspring are fertile as adults (**Figure 18.35a**). However, a P^- female does not produce the repressor protein, so none is stored in the cytoplasm of her eggs. Sperm contain little or no cytoplasm, so a P^+ male does not contribute the repressor protein to his offspring. When eggs from a P^- female are fertilized by sperm from a P^+ male, the absence of repression allows the *P* elements contributed by the sperm to undergo rapid transposition in the embryo, causing hybrid dysgenesis (**Figure 18.35b**). › **TRY PROBLEM 39**



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18.35 Hybrid dysgenesis in *Drosophila* is caused by the transposition of *P* elements.

Description

Part a on the left shows no hybrid dysgenesis. The P generation at the top shows a big P superscript negative male crossed with a big P superscript positive female. A caption reads, Cross a big P superscript negative male and a big P superscript positive female. Gamete production occurs below. The male produces a sperm with two slight V shaped, one blue tiny circular structure, and one rod shaped blue paternal chromosomes without P elements. The female produces an egg with two slight V shaped, one red tiny circular structure, and one rod shaped red Maternal chromosomes with yellow P elements at their bottom part. The egg also shows multiple repressors. A caption reads, repressor in the egg cytoplasm inhibits the transposition of P elements. Fertilization occurs and a zygote is obtained below. The zygote has two blue slight V shaped, one blue tiny circular structure,

and one rod shaped blue paternal chromosomes without P elements and two red slight V shaped, one tiny red circular structure, and one rod shaped red Maternal chromosomes with yellow P elements, separated by four repressors at the center. The F subscript 1 generation below shows a fertile fly. A caption reads, Thus, there is no hybrid dysgenesis and development is normal, producing fertile offspring.

The part b on the right shows Hybrid dysgenesis. The P generation at the top shows a big P superscript positive male crossed with a big P superscript negative female. A caption reads, Cross a big P superscript positive male and a big P superscript negative female. Gamete production occurs below. The male produces a sperm with two slight V shaped, one blue tiny circular structure, and one rod shaped blue paternal chromosomes with a yellow P elements at the their bottom parts. The female produces an egg with two slight V shaped, one red tiny circular structure, and one rod shaped red Maternal chromosomes without P elements. A caption reads, There is no repressor in the egg cytoplasm. Fertilization occurs and a zygote is obtained below. The zygote has two blue slight V shaped, one blue tiny circular structure, and one rod shaped blue paternal chromosomes with P elements and two red slight V shaped, one tiny red circular structure, and one rod shaped red Maternal chromosomes. Another zygote below has the same chromosomes but the red maternal chromosomes also have a P element at the bottom. The F subscript 1 generation below shows a sterile fly. A caption reads, Thus, P elements on the paternal chromosomes undergo a burst of transposition–hybrid dysgenesis, resulting in mutations, chromosome aberrations, and sterile offspring.

A caption below reads, Conclusion: Only the cross between a big P superscript positive male and a big P superscript negative female causes hybrid dysgenesis, because the sperm does not contribute a repressor.

TRANSPOSABLE ELEMENTS IN HUMANS

One of the most common transposable elements in the human genome is *Alu*. Every human cell contains more than 1 million related but not identical copies of *Alu* in its chromosomes. *Alu*

sequences are similar to those of the gene that encodes the 7S RNA molecule, which transports newly synthesized proteins across the endoplasmic reticulum. *Alu* sequences create short flanking direct repeats when they insert into DNA and have characteristics that suggest that they have transposed through an RNA intermediate.

Alu sequences belong to a class of repetitive sequences found frequently in mammalian and some other genomes. These sequences, collectively referred to as short interspersed elements (SINEs), constitute about 11% of the human genome. Most SINEs are copies of transposable elements that have been shortened at the 5′ end, probably because the reverse-transcription process used in their transposition terminated before the entire sequence was copied. SINEs have been identified as the cause of mutations in more than 20 cases of human genetic disease.

The human genome also has many transposons classified as long interspersed elements (LINEs), which are somewhat more similar in structure to retroviruses. Like SINEs, most LINEs in the human genome have been shortened at the 5′ end. The longest LINEs are usually about 6000 bp, but because most copies are shortened, the average LINE is only about 900 bp. There are approximately 900,000 copies of LINEs in the human genome, collectively constituting 21% of the total human DNA.

CONCEPTS

A great variety of transposable elements exist in eukaryotes. Some resemble transposable elements in prokaryotes, having terminal inverted repeats, and transpose as DNA. Others are retrotransposons that transpose through an RNA intermediate.

✔ CONCEPT CHECK 8

Hybrid dysgenesis results when

a. a male fly with *P* elements (P^+) mates with a female fly that lacks *P* elements (P^-).
b. a P^- male mates with a P^+ female.
c. a P^+ male mates with a P^+ female.
d. a P^- male mates with a P^- female.

CONNECTING CONCEPTS CONNECT

Types of Transposable Elements

Now that we have looked at some examples of transposable elements, let's review their major types (**Table 18.4**).

TABLE 18.4 Characteristics of two major classes of transposable genetic elements

	Structure	Genes Encoded	Transposition	Examples
Class I (retrotransposon)	Long terminal direct repeats; short flanking direct repeats at target site	Reverse-transcriptase gene (and sometimes others)	By RNA intermediate	*Ty* (yeast) *copia* (*Drosophila*) *Alu* (human)
Class II	Short terminal inverted repeats; short flanking	Transposase gene (and sometimes others)	Through DNA (replicative or nonreplicative)	*IS1* (*E. coli*) *Tn3* (*E. coli*)

	direct repeats at target site			*Ac, Ds* (maize) *P* elements (*Drosophila*)

Transposable elements can be divided into two major classes on the basis of their structure and movement. Class I comprises the retrotransposons, which possess terminal direct repeats and transpose through RNA intermediates. They generate flanking direct repeats at their points of insertion when they transpose into DNA. Retrotransposons do not encode transposase, but some types are similar in structure to retroviruses and carry sequences that produce reverse transcriptase. Transposition takes place when transcription produces an RNA intermediate, which is then transcribed into DNA by reverse transcriptase and inserted into the target site. Examples of retrotransposons include *Ty* elements in yeast and *Alu* sequences in humans. Retrotransposons are not found in prokaryotes.

Class II consists of DNA transposons that possess terminal inverted repeats and transpose as DNA. Like Class I transposons, they generate flanking direct repeats at their points of insertion into DNA. Unlike Class I transposons, all active forms of Class II transposable elements encode transposase, which is required for their movement. Some also encode resolvase, repressors, and other proteins. Their transposition may be replicative or nonreplicative, but they never use RNA intermediates. Examples of transposable elements in this class include insertion sequences and all complex transposons in bacteria, *Ac* and *Ds* elements in corn, and *P* elements in *Drosophila*.

Transposable Elements in Genome Evolution

Transposable elements have clearly played an important role in shaping the genomes of many organisms. About 50% of all spontaneous mutations in *Drosophila*, for example, are due to

transposition, and much of the tremendous variation in genome size found among eukaryotic organisms is due to differences in numbers of copies of transposable elements. Homologous recombination between copies of transposable elements has been an important force in producing gene duplications and other chromosome rearrangements. Furthermore, some transposable elements may carry extra DNA with them when they transpose to a new site, providing the potential to move DNA sequences that regulate genes to new sites, where they may alter the expression of genes.

TRANSPOSABLE ELEMENTS AS GENOMIC PARASITES

As we have seen, transposable elements that undergo replicative transposition leave a copy behind when they transpose to a new location and therefore increase in number within a genome with the passage of time. This ability to replicate and spread suggests that many transposable elements serve no purpose for the cell; they exist simply because they are capable of replicating and spreading. The insertion of transposable elements into a gene often destroys its function, with harmful consequences for the cell. Furthermore, expenditure of the time and energy required to replicate large numbers of transposable elements is likely to place a metabolic burden on the cell. Thus, transposable elements can be thought of as genomic parasites that provide no benefit to the cell and may even be harmful.

DOMESTICATION OF TRANSPOSABLE ELEMENTS

Although many transposable elements may be genomic parasites, some have clearly evolved to serve useful purposes for their host cells. These transposons are sometimes referred to as domesticated, implying that their parasitic tendencies have been replaced by properties useful to the cell. For example, the mechanism that generates antibody diversity in the immune systems of vertebrates (see Chapter 22) probably evolved from a transposable element. Immune-system cells called lymphocytes have the ability to unite several DNA segments that encode antigen-recognition proteins. This mechanism may have arisen from a transposable element that inserted into the germ line of a vertebrate ancestor some 450 million years ago.

Transposable elements have also played an important role in the evolution of corn. Modern corn (maize) was domesticated from teosinte in Central America more than 8000 years ago. One of the important genetic differences between teosinte and corn involves a gene called *tb1*, which encodes a transcriptional regulator that represses the growth of side branches. In corn, transcription of *tb1* is elevated compared with that in teosinte, with the result that modern corn is more upright and less branched than teosinte. The elevated transcription of *tb1* in corn is the result of a transposable element called *Hopscotch* that inserted into regulatory sequences that control *tb1* transcription. This insertion was probably present in teosinte as a variant and was selected by humans during the

domestication of corn because it produced a more desirable plant shape. Transposable elements may also play a role in speciation, the process by which new species arise (see the earlier discussion of hybrid dysgenesis).

THINK-PAIR-SHARE Question 6

CONCEPTS

Many transposable elements appear to be genomic parasites, existing in large numbers because of their ability to increase in copy number. Such increases in copy numbers of transposable elements have contributed to the large size of many eukaryotic genomes. In several cases, transposable elements have become adapted for specific cellular functions.

18.5 A Number of Pathways Can Repair DNA

The integrity of DNA is under constant assault from radiation, chemical mutagens, and spontaneously arising changes. In spite of these damaging agents, the rate of mutation remains remarkably low, thanks to the efficiency with which DNA is repaired.

There are a number of complex pathways for repairing DNA, but several general statements can be made about DNA repair. First, most DNA-repair mechanisms require two nucleotide strands of DNA because most replace whole nucleotides, and a template strand is needed to specify the base sequence.

A second general feature of DNA repair is redundancy, meaning that many types of DNA damage can be corrected by more than one repair system. This redundancy illustrates the extreme importance of DNA repair to the survival of the cell: if a mistake escapes one repair system, it's likely to be repaired by another system, ensuring that almost all mistakes are corrected.

Much research has been conducted on mechanisms of DNA repair, and the 2015 Nobel Prize in chemistry was awarded to Tomas Lindahl, Paul Modrich, and Aziz Sancar for their pioneering work on DNA repair. Here we consider five general mechanisms of DNA

repair: mismatch repair, direct repair, base-excision repair, nucleotide-excision repair, and repair of double-strand breaks.

Mismatch Repair

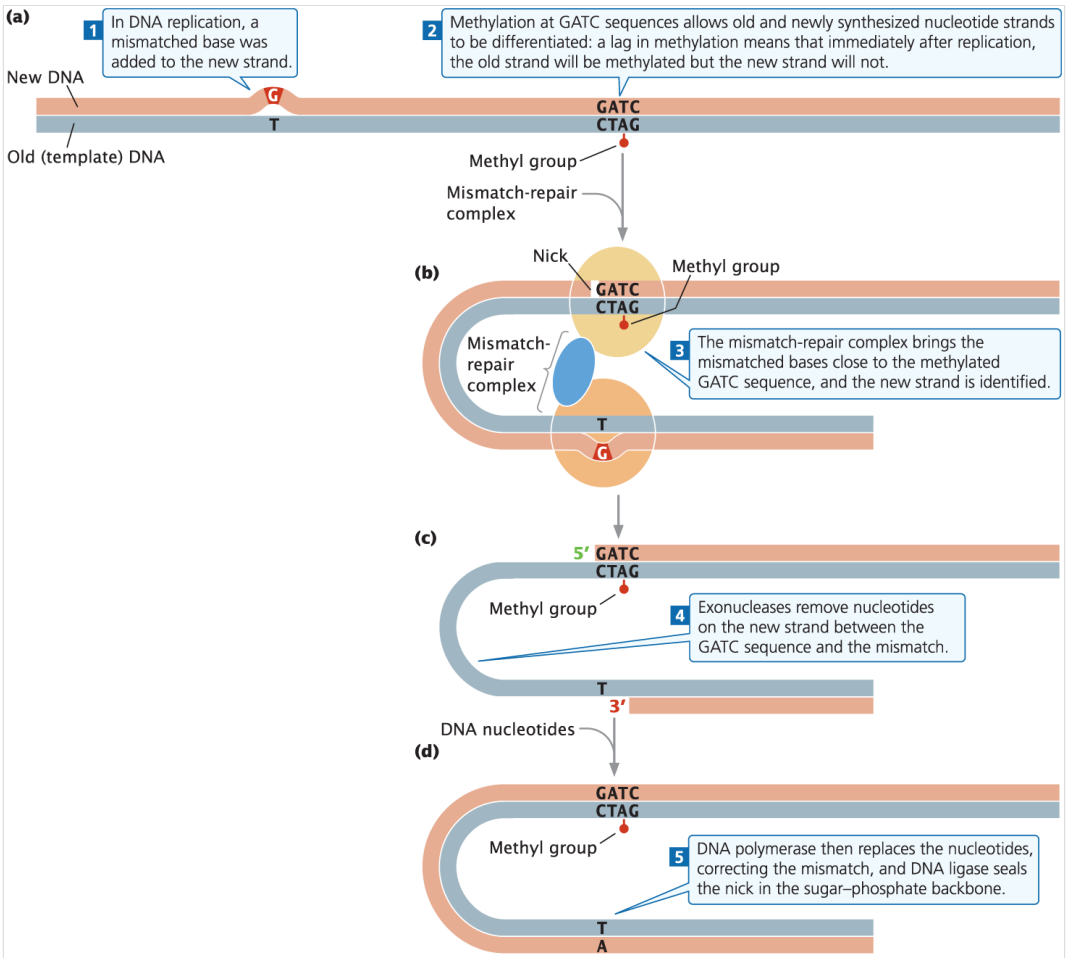
Replication is extremely accurate: each new copy of DNA has less than one error per billion nucleotides. However, in the process of replication, mismatched bases are incorporated into the new DNA with a frequency of about 10^{-4} to 10^{-5}; this discrepancy shows that most of the errors that initially arise are corrected and never become permanent mutations. Some of these corrections are made in proofreading (see p. 359 in Chapter 12) by the DNA polymerases.

Many incorrectly inserted nucleotides that escape detection by proofreading are corrected by *mismatch repair*, in which incorrectly paired bases are detected and corrected by mismatch-repair enzymes. In addition, the mismatch-repair system corrects small unpaired loops in the DNA, such as those caused by strand slippage in replication (see Figure 18.13). However, some nucleotide repeats may form secondary structures on the unpaired strand (see Figure 18.5), allowing them to escape detection by the mismatch-repair system.

After an incorporated error has been recognized, the mismatch-repair enzymes cut out a section of the newly synthesized strand and fill the gap with new nucleotides by using the original DNA strand as

a template. For this strategy to work, mismatch repair must have some way of distinguishing between the old and the new strands of the DNA so that the incorporated error, but not part of the original strand, is removed.

The proteins that carry out mismatch repair in *E. coli* differentiate between old and new strands by the presence of methyl groups on certain sequences of the old strand. After replication, adenine nucleotides in the sequence GATC are methylated. The process of methylation is delayed, however, so immediately after replication, the old strand is methylated and the new strand is not (**Figure 18.36a**). The mismatch-repair complex brings an unmethylated GATC sequence close to the mismatched bases. It nicks the unmethylated strand at the GATC site (**Figure 18.36b**) and degrades the strand between the nick and the mismatched bases (**Figure 18.36c**). DNA polymerase and DNA ligase fill in the gap on the unmethylated strand with correctly paired nucleotides (**Figure 18.36d**).



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18.36 Many incorrectly inserted nucleotides that escape proofreading are corrected by mismatch repair.

Description

Part a at the top shows a double D N A strand. The top strand is labeled new D N A and has a mismatched nucleotide G looping out on the left side. A caption reads, In D N A replication, a mismatched base was added to the new strand. The sequence on the right side is as follows: G, A, T, C. A caption reads, Methylation at G A T C sequences allows old and newly synthesized nucleotide strands to be differentiated: a lag in methylation means that immediately after replication, the old strand will be methylated but the new strand will not.

A strand below labeled old (template) strand shows nucleotide T against the looped out G on the above D N A strand. The sequence on the right side is as follows: C, T, A with a

methyl group below, G. A mismatch repair complex enters the process.

Part b on the right side shows bending of D N A strands on the left side such that the looped out part is now at the bottom and the other groups are at the top. A nick is formed before the G, A, T, C nucleotides of new strand. The mismatch repair complex brings the methylated nucleotides and looped out nucleotide close. A caption reads, The mismatch-repair complex brings the mismatched bases close to the methylated G A T C sequence, and the new strand is identified.

Part c below shows the area between mismatch sequence and the G, A, T, C nucleotide is removed, from the new strand while both strands are still bent on the left. A caption reads, Exonucleases remove nucleotides on the new strand between the G A T C sequence and the mismatch. D N A nucleotides are added.

Part d below shows formation of the strand missing on the new strand while both strands are still bent on the left. The new strand now contains A nucleotide corresponding to the T nucleotide on the old strand. A caption reads, D N A polymerase then replaces the nucleotides, correcting the mismatch, and D N A ligase seals the nick in the sugar-phosphate backbone.

Text on the bottom left reads, the mismatch-repair system corrects small unpaired loops in the D N A, such as those caused by strand slippage in replication (see Figure 18 point 13). However, some nucleotide repeats may form secondary structures on the unpaired strand (see Figure 18 point 5), allowing them to escape detection by the mismatch-repair system. After an incorporated error has been recognized, the mismatch-repair enzymes cut out a section of the newly synthesized strand and fill the gap with new nucleotides by using the original D N A strand as a template. For this strategy to work, mismatch repair must have some way of distinguishing between the old and the new strands of the D N A so that the incorporated error, but not part of the original strand, is removed. The proteins that carry out mismatch repair in E. coli differentiate between old and new strands by the presence of methyl groups on certain sequences of the old strand. After replication, adenine nucleotides in the sequence G A T C are methylated. The process of methylation is delayed, however, so immediately after replication, the old strand is methylated and the new strand is not.

Mismatch repair in eukaryotic cells is similar to that in *E. coli*, but how the old and new strands are recognized in eukaryotic cells is not known. In some eukaryotes, such as yeast and fruit flies, there is no detectable methylation of DNA, and yet mismatch repair still takes place. Humans who possess mutations in mismatch-repair genes often exhibit elevated somatic mutation rates and are frequently susceptible to colon cancer.

CONCEPTS

Mismatched bases and other DNA lesions are corrected by mismatch repair. Enzymes cut out a section of the newly synthesized strand of DNA and replace it with new nucleotides.

✔ CONCEPT CHECK 9

Mismatch repair in *E. coli* distinguishes between old and new strands of DNA on the basis of

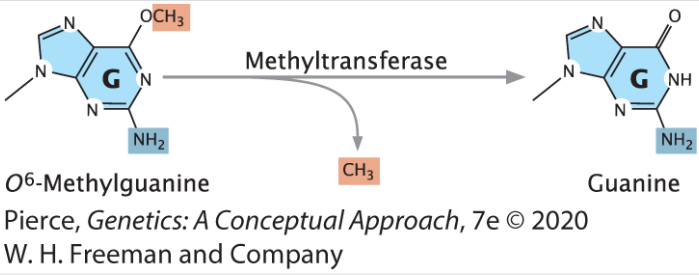
a. differences in the base composition of the two strands.
b. modification of histone proteins.
c. base analogs on the new strand.
d. methyl groups on the old strand.

Direct Repair

Direct repair does not replace altered nucleotides but instead restores their original (correct) structures. One of the best-understood direct-repair mechanisms is the photoreactivation of UV-

induced pyrimidine dimers (see Figure 18.22). *Escherichia coli* and some eukaryotic cells possess an enzyme called photolyase, which uses energy captured from light to break the covalent bonds that link the pyrimidines in a dimer.

Direct repair also corrects O^6-methylguanine, an alkylation product of guanine that pairs with adenine, producing G • C → T • A transversions. An enzyme called O^6-methylguanine-DNA methyltransferase removes the methyl group from O^6-methylguanine, restoring the base to guanine (**Figure 18.37**).

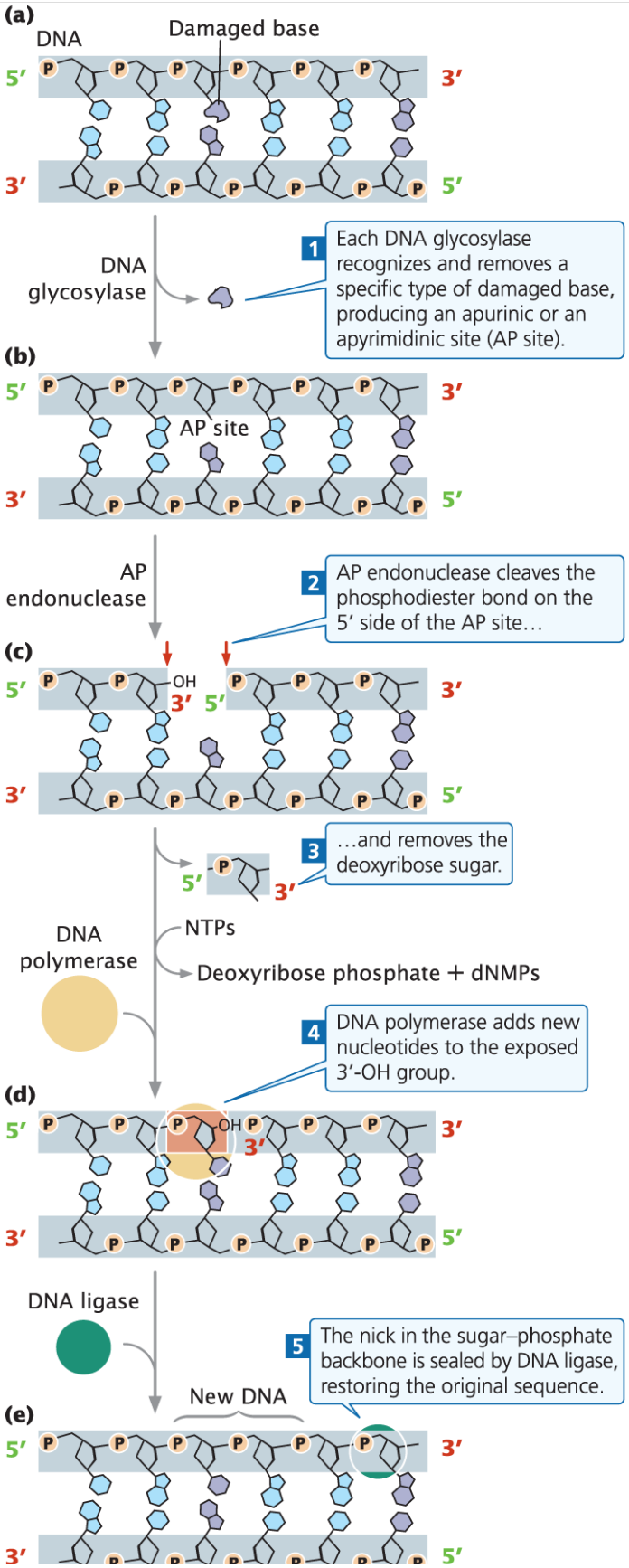


18.37 Direct repair restores the original structures of nucleotides.

Description
The illustration shows O superscript 6-Methylguanine with methyl group at C 6 highlighted and amino group at C 2 highlighted. A forward arrow representing Methyltransferase removes methyl group. Guanine is obtained on the right with the amino group at C 2 highlighted.

Base-Excision Repair

In **base-excision repair**, a modified base is excised, and then the entire nucleotide is replaced. The excision of modified bases is catalyzed by a set of enzymes called DNA glycosylases, each of which recognizes and removes a specific type of modified base by cleaving the bond that links that base to the 1′-carbon atom of a deoxyribose sugar (**Figure 18.38a**). Uracil glycosylase, for example, recognizes and removes uracil produced by the deamination of cytosine. Other glycosylases recognize hypoxanthine, 3-methyladenine, 7-methylguanine, and other modified bases.


(a)
DNA
Damaged base
5′
3′
3′
5′
DNA glycosylase
1 Each DNA glycosylase recognizes and removes a specific type of damaged base, producing an apurinic or an apyrimidinic site (AP site).
(b)
AP site
AP endonuclease
2 AP endonuclease cleaves the phosphodiester bond on the 5′ side of the AP site…
(c)
OH
3 …and removes the deoxyribose sugar.
DNA polymerase
NTPs
Deoxyribose phosphate + dNMPs
4 DNA polymerase adds new nucleotides to the exposed 3′-OH group.
(d)
DNA ligase
5 The nick in the sugar–phosphate backbone is sealed by DNA ligase, restoring the original sequence.
New DNA
(e)

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18.38 Base-excision repair excises modified bases and then replaces one or more nucleotides.

Description

Part a, at the top, shows a D N A double strand. The top strand runs 5 prime to 3 prime and the bottom strand runs 3 prime to 5 prime. The sugar molecules in the both strands are joined to bases at their C 1 and are joined to phosphate groups at C 4. A damaged base is present at third sugar molecule from left on the top strand. D N A glycosylase removes the damaged base. A caption reads, Each D N A glycosylase recognizes and removes a specific type of damaged base, producing an apurinic or an apyrimidinic site (A P site).

Part b below shows a D N A double strand. The top strand runs 5 prime to 3 prime and the bottom strand runs 3 prime to 5 prime. The sugar molecules in the both strands are joined to bases at their C 1 and are joined to phosphate groups at C 4. An A P site is present at third sugar molecule from left on the top strand. A P endonuclease enters the process.

Part c below shows a D N A double strand. The top strand runs 5 prime to 3 prime and the bottom strand runs 3 prime to 5 prime. The sugar molecules in the both strands are joined to bases at their C 1 and are joined to phosphate groups at C 4. A phosphodiester bond attached to the third sugar molecule from left on the top strand is broken and is eliminated along with sugar molecule. The first cut site now has and O H group and shows 3 prime polarity. The other cut shows 5 prime polarity. A caption reads, A P endonuclease cleaves the phosphodiester bond on the 5 prime side of the A P site, and removes the deoxyribose sugar. N T P's converts to Deoxyribose phosphate plus d N M P's. D N A polymerase enters the process.

Part d below shows a D N A double strand. The top strand runs 5 prime to 3 prime and the bottom strand runs 3 prime to 5 prime. The sugar molecules in the both strands are joined to bases at their C 1 and are joined to phosphate groups at C 4. The third sugar molecule from left on the first strand is highlighted and a caption reads, D N A polymerase adds new nucleotides to the exposed 3 prime O H group. D N A ligase enters the process.

Part e below shows a D N A double strand. The top strand runs 5 prime to 3 prime and the bottom strand runs 3 prime to 5 prime. The sugar molecules in the both strands are joined to bases at their C 1 and are joined to phosphate groups at C 4. The top strand is labeled new D N A. A caption reads, The nick in the sugar-phosphate backbone is sealed by D N A ligase, restoring the original sequence.

After the modified base has been removed from a nucleotide, an enzyme called AP (apurinic or apyrimidinic) endonuclease cuts the phosphodiester bond, and other enzymes remove the deoxyribose sugar (**Figure 18.38b**). DNA polymerase then adds one or more new nucleotides to the exposed 3′-OH group (**Figure 18.38c**), replacing a section of nucleotides on the damaged strand. The nick in the sugar–phosphate backbone is sealed by DNA ligase (**Figure 18.38d**), and the original intact sequence is restored (**Figure 18.38e**).

Bacteria use DNA polymerase I to replace excised nucleotides, but eukaryotes use DNA polymerase *β*, which has no proofreading ability and tends to make mistakes. On average, DNA polymerase *β* makes one mistake per 4000 nucleotides inserted. About 20,000 to 40,000 base modifications per day are repaired by base excision, so DNA polymerase *β* may introduce as many as 10 errors per day into the human genome. How are these errors corrected? Recent research results show that some AP endonucleases have the ability to proofread. When DNA polymerase *β* inserts a nucleotide with the wrong base into the DNA, DNA ligase cannot seal the nick in the sugar–phosphate backbone because the 3′-OH and 5′-phosphate groups of adjacent nucleotides are not in the correct orientation for

the ligase to connect them. In this case, AP endonuclease 1 detects the mispairing and uses its $3' \rightarrow 5'$ exonuclease activity to excise the incorrectly paired base. DNA polymerase β then uses its polymerase activity to fill in the missing nucleotide. In this way, the fidelity of base-excision repair is maintained.

CONCEPTS

Direct-repair mechanisms restore the correct structures of altered nucleotides. In base-excision repair, glycosylase enzymes recognize and remove specific types of modified bases. The entire nucleotide is then removed and a section of the polynucleotide strand is replaced.

✔ CONCEPT CHECK 10

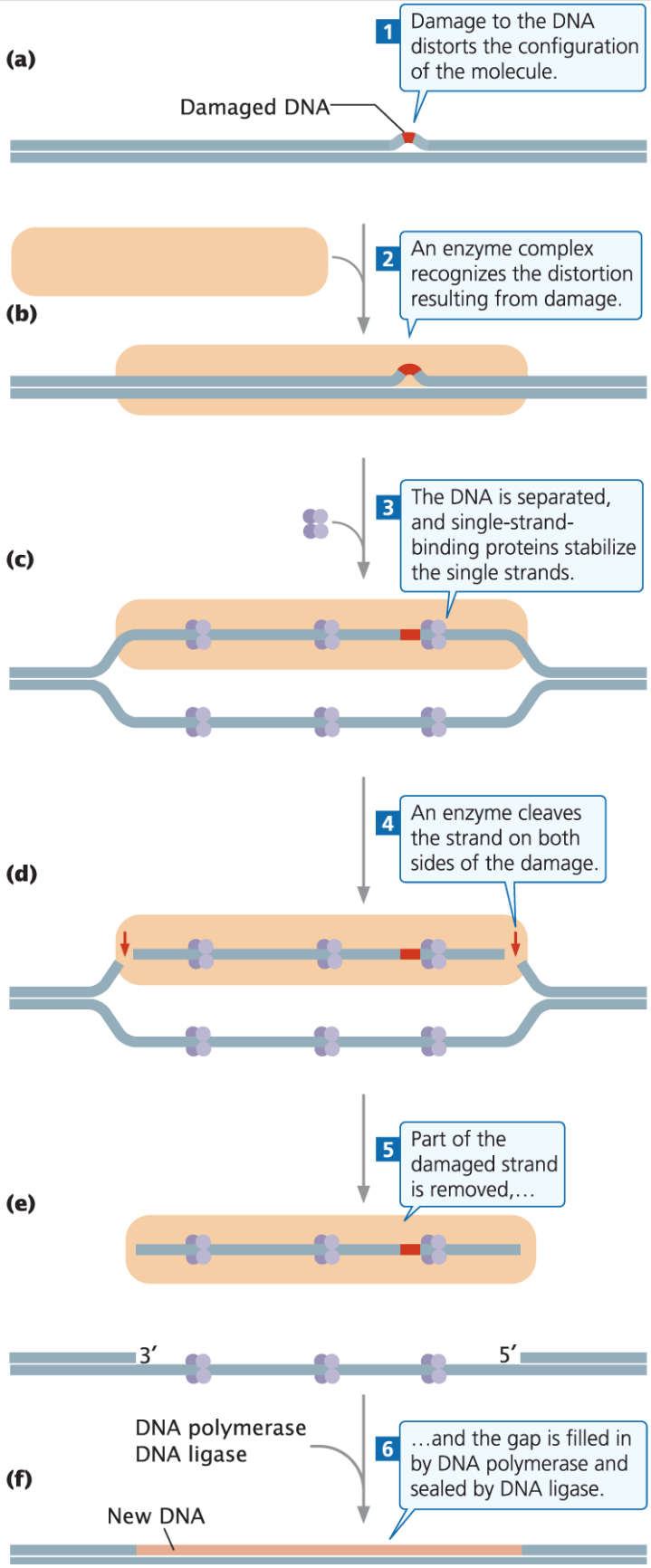
How does direct repair differ from mismatch repair and base-excision repair?

Nucleotide-Excision Repair

Another DNA-repair pathway is **nucleotide-excision repair**, which removes bulky DNA lesions (such as pyrimidine dimers) that distort the double helix. Nucleotide-excision repair can repair many different types of DNA damage and is found in cells of all organisms, from bacteria to humans.

The process of nucleotide-excision repair is complex; in humans, a large number of genes take part. First, a complex of enzymes scans

DNA, looking for distortions of its three-dimensional configuration (**Figure 18.39a and b**). When a distortion is detected, additional enzymes separate the two nucleotide strands at the damaged region, and single-strand-binding proteins stabilize the separated strands (**Figure 18.39c**). Next, the sugar–phosphate backbone of the damaged strand is cleaved on both sides of the damage (**Figure 18.39d**). Part of the damaged strand is peeled away by helicase enzymes (**Figure 18.39e**), and the gap is filled in by DNA polymerase and sealed by DNA ligase (**Figure 18.39f**).


(a)
1 Damage to the DNA distorts the configuration of the molecule.
Damaged DNA
(b)
2 An enzyme complex recognizes the distortion resulting from damage.
(c)
3 The DNA is separated, and single-strand-binding proteins stabilize the single strands.
(d)
4 An enzyme cleaves the strand on both sides of the damage.
(e)
5 Part of the damaged strand is removed,…
3′
5′
(f)
DNA polymerase
DNA ligase
6 …and the gap is filled in by DNA polymerase and sealed by DNA ligase.
New DNA

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18.39 Nucleotide-excision repair removes bulky DNA lesions that distort the double helix.

Description

Part a, at the top, shows a D N A double strand. The top strand shows a loop toward the right side and is labeled damaged D N A. A caption reads, Damage to the D N A distorts the configuration of the molecule. A large enzyme complex enters the process.

Part b below shows enzyme complex binds to the D N A double strand. A caption reads, An enzyme complex recognizes the distortion resulting from damage.

Part c below shows single-strand binding proteins enter the process. The double strand unwinds from the center. The enzyme complex is still bound to the top strand with damaged D N A. Three single-strand binding proteins attach to each of the strands. A caption reads, The D N A is separated, and single-strand binding proteins stabilize the single strands.

Part d below shows the area of the top strand with damaged D N A is excised from the either side of the enzyme complex. The single strand binding proteins are still attached to the bottom strand and the cleaved strand. A caption reads, An enzyme cleaves the strand on both sides of the damage.

Part e below shows the cleaved strand is removed along with the enzyme complex. The remaining top strand with a removed region is paired with bottom strand which is still stabilized by single strand binding proteins. The first cut on the top strand shows polarity 3 prime and the other end shows 5 prime. A caption reads, Part of the damaged strand is removed. D N A polymerase and D N A ligase enter the process.

Part f below shows a double stranded D N A with a new strand filling the gap in the top strand. A caption reads, the gap is filled in by D N A polymerase and sealed by D N A ligase.

CONCEPTS

Nucleotide-excision repair removes and replaces many types of damaged DNA that distort the DNA structure. The two strands of DNA are separated, a section of the DNA containing the distortion is removed, DNA polymerase fills in the gap, and DNA ligase seals the filled-in gap.

CONNECTING CONCEPTS

The Basic Pathway of DNA Repair

We have now examined several different mechanisms of DNA repair. What do these mechanisms have in common? How are they different? Most mechanisms of DNA repair depend on the presence of two strands because nucleotides in the damaged area are removed and replaced. Nucleotides are replaced in mismatch repair, base-excision repair, and nucleotide-excision repair, but are not replaced by direct-repair mechanisms.

Repair mechanisms that include nucleotide removal use a common four-step pathway:

1. Detection: The damaged section of the DNA is recognized.
2. Excision: Endonucleases nick the sugar–phosphate backbone on one or both sides of the DNA damage, and one or more nucleotides are removed.
3. Polymerization: DNA polymerase adds nucleotides to the newly exposed $3'$-OH group by using the other strand as a template and replacing damaged nucleotides (and frequently some undamaged ones as well).
4. Ligation: DNA ligase seals the nicks in the sugar–phosphate backbone.

The primary differences in the mechanisms of mismatch, base-excision, and nucleotide-excision repair are in the details of detection and excision. In base-excision and mismatch repair, a single nick is made in the sugar–phosphate backbone on one side of the damage; in nucleotide-excision repair, nicks are made on both sides of the DNA lesion. In base-excision repair, DNA polymerase displaces the old nucleotides as it adds new nucleotides to the $3'$ end of the nick; in mismatch repair, the old nucleotides are degraded; and in nucleotide-excision repair, the old nucleotides are displaced by helicase enzymes. All three mechanisms use DNA polymerase and ligase to fill in the gap produced by the excision and removal of damaged nucleotides.

Repair of Double-Strand Breaks

A common type of DNA damage is a double-strand break, in which both strands of the DNA helix are broken. Double-strand breaks are caused by ionizing radiation, oxidative radicals, and other DNA-damaging agents. These types of breaks are particularly detrimental to the cell because they stall DNA replication and may lead to chromosome rearrangements, such as deletions, duplications, inversions, and translocations. There are two major pathways for repairing double-strand breaks: homology directed repair and nonhomologous end joining.

HOMOLOGY DIRECTED REPAIR

Homology directed repair (also known as homologous recombination) repairs a broken DNA molecule by using the identical or nearly identical genetic information contained in another DNA molecule, usually a sister chromatid, the same mechanism employed in the process of homologous recombination that is responsible for crossing over (see Chapter 12). Homology directed repair begins with the removal of some nucleotides at the broken ends, followed by strand invasion, displacement, and replication (see Figure 12.20). Many of the same enzymes that carry out crossing over are used in the repair of double-strand breaks by homology directed repair: two such enzymes are BRCA1 and BRCA2.

The genes that encode these proteins are frequently mutated in breast-cancer cells.

NONHOMOLOGOUS END JOINING

Nonhomologous end joining repairs double-strand breaks without using a homologous template. This pathway is often used when the cell is in G_1 and a sister chromatid is not available for repair through homology directed repair. Nonhomologous end joining uses proteins that recognize the broken ends of DNA, bind to the ends, and then join them together. Nonhomologous end joining is more error prone than homology directed repair and often leads to deletions, insertions, and translocations. A poorly understood alternative type of end joining is microhomology-mediated end joining (MMEJ), which relies on very small (5–25 bp) homologies near the ends of broken DNA molecules to recognize the ends and rejoin them. Because the regions of homology are so short, this mechanism is also more error prone than homology directed repair. Nonhomologous end joining plays an important role in somatic recombination, a process that leads to the antibody diversity in the immune system (see Chapter 22).The most common DNA-repair mechanisms are summarized in **Table 18.5**.

TABLE 18.5 Summary of common DNA-repair mechanisms

Repair System	Type of Damage Repaired
Mismatch	Replication errors, including mispaired bases and strand slippage
Direct	Pyrimidine dimers; other specific types of alterations

Base excision	Abnormal bases, modified bases, and pyrimidine dimers
Nucleotide excision	DNA damage that distorts the double helix, including abnormal bases, modified bases, and pyrimidine dimers
Homology directed repair	Double-strand breaks
Nonhomologous end joining	Double-strand breaks

Translesion DNA Polymerases

As discussed in Chapter 12, the high-fidelity DNA polymerases that normally carry out replication operate at high speed and, like a high-speed train, require a smooth track—an undistorted template. Some mutations, such as pyrimidine dimers, produce distortions in the three-dimensional structure of the DNA helix, blocking replication by the high-speed polymerases. When distortions of the template are encountered, specialized translesion DNA polymerases take over replication and bypass the lesions.

The translesion polymerases are able to bypass bulky lesions but, in the process, often make errors. Thus, the translesion polymerases allow replication to proceed at the cost of introducing errors into the sequence. Some of these errors are corrected by DNA-repair systems, but others escape detection.

An example of a translesion DNA polymerase is polymerase η (eta), which bypasses pyrimidine dimers in eukaryotes. Polymerase η

inserts AA opposite a pyrimidine dimer. This strategy seems to be reasonable because about two-thirds of pyrimidine dimers are thymine dimers. However, the insertion of AA opposite a CT dimer results in a C • G → T • A transversion. Polymerase η therefore tends to introduce mutations into the DNA sequence.

CONCEPTS

Two major pathways exist for the repair of double-strand breaks in DNA: homology directed repair and nonhomologous end joining. Translesion DNA polymerases allow replication to proceed past bulky distortions in the DNA but often introduce errors as they bypass the distorted region.

Genetic Diseases and Faulty DNA Repair

Several human diseases are connected to defects in DNA repair. These diseases are often associated with high incidences of specific cancers because defects in DNA repair lead to increased rates of mutation. This phenomenon is discussed further in Chapter 23.

Among the best studied of the human DNA-repair diseases is xeroderma pigmentosum (**Figure 18.40**), a rare autosomal recessive condition that includes abnormal skin pigmentation and acute sensitivity to sunlight. People who have this disease also have a

strong predisposition to skin cancer, with an incidence ranging from 1000 to 2000 times that found in unaffected people. Sunlight includes a strong UV component, so exposure to sunlight produces pyrimidine dimers in the DNA of skin cells. Although human cells lack photolyase (the enzyme that repairs pyrimidine dimers in bacteria), most pyrimidine dimers in humans can be corrected by nucleotide-excision repair (see Figure 18.39). However, the cells of most people with xeroderma pigmentosum have defective nucleotide-excision repair, so many of their pyrimidine dimers go uncorrected and may lead to cancer.

Stephane AUDRAS/REA/Redux.

18.40 Xeroderma pigmentosum results from defects in DNA repair. The disease is characterized by freckle-like spots on the skin (shown here) and a predisposition to skin cancer.

Xeroderma pigmentosum can result from defects in several different genes. Some individuals with xeroderma pigmentosum have mutations in a gene encoding the protein that recognizes and binds to damaged DNA; others have mutations in a gene encoding helicase.

Still others have defects in the genes whose products play a role in cutting the damaged strand on the 5′ or 3′ sides of the pyrimidine dimer. Some people have a slightly different form of the disease (xeroderma pigmentosum variant) owing to mutations in the gene encoding polymerase η, the translesion DNA polymerase that bypasses pyrimidine dimers.

Another genetic disease caused by faulty DNA repair is an inherited form of colon cancer called hereditary nonpolyposis colon cancer (HNPCC). It is one of the most common hereditary cancers, accounting for about 15% of colon cancers. Research findings indicate that HNPCC arises from mutations in the proteins that carry out mismatch repair (see Figure 18.36). Some genetic diseases associated with defective DNA repair are listed in **Table 18.6**.

› **TRY PROBLEM 45**

THINK-PAIR-SHARE Question 7

TABLE 18.6 Genetic diseases associated with defects in DNA-repair systems

Disease	Symptoms	Genetic Defect
Xeroderma pigmentosum	Freckle-like spots on skin, sensitivity to sunlight,	Defects in nucleotide-excision repair

	predisposition to skin cancer	
Cockayne syndrome	Dwarfism, sensitivity to sunlight, premature aging, deafness, intellectual disability	Defects in nucleotide-excision repair
Trichothiodystrophy	Brittle hair, skin abnormalities, short stature, immature sexual development, characteristic facial features	Defects in nucleotide-excision repair
Hereditary nonpolyposis colon cancer	Predisposition to colon cancer	Defects in mismatch repair
Fanconi anemia	Increased skin pigmentation; abnormalities of skeleton, heart, and kidneys;	Possibly defects in the repair of interstrand cross-links

	predisposition to leukemia	
Li-Fraumeni syndrome	Predisposition to cancer in many different tissues	Defects in DNA damage response
Werner syndrome	Premature aging, predisposition to cancer	Defects in homology directed repair

CONCEPTS

Defects in DNA repair are the underlying cause of several genetic diseases. Many of these diseases are characterized by a predisposition to cancer.

✔ CONCEPT CHECK 11

Why are defects in DNA repair often associated with increases in cancer?

Chapter 18 Review

CHAPTER SUMMARY

Section 18.1 Learning Objective: Be able to outline the importance of mutations and the main categories of mutations.

- Mutations are heritable changes in genetic information. Somatic mutations occur in somatic cells; germ-line mutations occur in cells that give rise to gametes.
- The simplest type of mutation is a base substitution, a change in a single base pair of DNA. Transitions are base substitutions in which purines are replaced by purines or pyrimidines are replaced by pyrimidines. Transversions are base substitutions in which a purine replaces a pyrimidine or a pyrimidine replaces a purine.
- Insertions are additions of nucleotides, and deletions are removals of nucleotides. These mutations often change the reading frame of the gene.
- Expanding nucleotide repeats are mutations in which the number of copies of a set of nucleotides increases with the passage of time; they are responsible for several human genetic diseases.
- A missense mutation alters the coding sequence so that one amino acid is substituted for another. A nonsense mutation changes a codon that specifies an amino acid into a stop codon. A silent mutation produces a synonymous codon that specifies the same amino acid as the original sequence, whereas a neutral mutation alters the amino acid sequence but does not change the functioning of the protein.
- A suppressor mutation reverses the effect of a previous mutation at a different site and may be intragenic (within the same gene as the original mutation) or intergenic (within a different gene).
- The mutation rate is the frequency with which a particular mutation arises. Mutation rates are influenced by both genetic and environmental factors.

Section 18.2 Learning Objective: Be able to outline the different ways that gene mutations may arise.

- Some mutations occur spontaneously. These mutations include the mispairing of bases in replication and spontaneous depurination and deamination.
- Insertions and deletions can arise from strand slippage in replication or from unequal crossing over.
- Base analogs can become incorporated into DNA in the course of replication and pair with the wrong base in subsequent replication events. Alkylating agents, deaminating chemicals, and hydroxylamine lead to mutations by modifying the chemical structure of bases. Intercalating agents insert themselves into the DNA molecule and cause single-nucleotide insertions and deletions. Oxidative reactions alter the chemical structures of bases.

Section 18.3 Learning Objective: Be able to explain how mutations have been detected and studied.

- Ionizing radiation is mutagenic, altering base structures and breaking phosphodiester bonds. Ultraviolet light produces pyrimidine dimers, which block replication. Bacteria use the SOS system to overcome replication blocks produced by pyrimidine dimers and other lesions in DNA.
- The Ames test uses bacteria to assess the mutagenic potential of chemical substances.

Section 18.4 Learning Objective: Be able to describe the features and effects of transposable elements.

- Transposable elements are mobile DNA sequences that insert into many locations within a genome and often cause mutations and DNA rearrangements.
- Most transposable elements have two common characteristics: terminal inverted repeats and short flanking direct repeats generated at the point of insertion.
- Transposable elements may transpose as DNA or through the production of an RNA intermediate that is then reverse transcribed into DNA. In replicative transposition, the transposable element is copied and the copy moves to a new site; in nonreplicative transposition, the transposable element excises from the old site and moves to a new site.
- Insertion sequences are small bacterial transposable elements that carry only the information needed for their own movement. Composite transposons in bacteria are more complex elements that consist of DNA between two insertion sequences.

Some complex transposable elements in bacteria do not contain insertion sequences.

- DNA transposons in eukaryotic cells are similar to those found in bacteria, ending in short inverted repeats and producing flanking direct repeats at the point of insertion. Other eukaryotic transposons are retrotransposons, similar in structure to retroviruses, which transpose through RNA intermediates.
- Transposons have played an important role in genome evolution.

Section 18.5 Learning Objective: Be able to describe different pathways of DNA repair.

- Most damage to DNA is corrected by DNA-repair mechanisms. These mechanisms include mismatch repair, direct repair, base-excision repair, and nucleotide-excision repair. Most repair pathways require two strands of DNA. The various pathways exhibit some redundancy in the types of damage repaired.
- Double-strand breaks are repaired by homology directed repair and nonhomologous end joining. Translesion DNA polymerases allow replication to proceed past bulky distortions in the DNA.
- Defects in DNA repair are the underlying cause of several genetic diseases.

WORKED PROBLEMS

Problem 1

The mutations produced by the following numbered compounds can be reversed by the substances shown. What conclusions can you draw about the nature of the mutations originally produced by these compounds?

	Mutations produced by compound	Reversed by			
		5-Bromouracil	EMS	Hydroxyl-amine	Acridine orange
a.	1	Yes	Yes	Yes	No
b.	2	Yes	Yes	Some	No

c.	3	No	No	No	Yes
d.	4	Yes	Yes	Yes	Yes

Solution Strategy

What information is required in your answer to the problem?

The types of mutations produced by each compound.

What information is provided to solve the problem?

Which substances reverse the mutations produced by each compound.

For help with this problem, review:

Chemically Induced Mutations in Section 18.2.

Solution Steps

a. Mutations produced by compound 1 are reversed by 5-bromouracil, which produces both A • T → G • C and G • C → A • T transitions, which tells us that compound 1 produces single-base substitutions that may include the generation of either A • T or G • C base pairs. The mutations produced by compound 1 are also reversed by EMS, which, like 5-bromouracil, produces both A • T → G • C and G • C → A • T transitions, so no additional information is provided here. Hydroxylamine does not reverse the mutations produced by compound 1. Because hydroxylamine produces only C • G → T • A transitions, we know that compound 1 does not generate C • G base pairs. Acridine orange, an intercalating agent that produces frameshift mutations, also does not reverse the mutations, revealing that compound 1 produces only single-base substitutions, not insertions or deletions. In summary, compound 1 appears to cause single-base substitutions that generate T • A but not G • C base pairs.

b. Compound 2 generates mutations that are reversed by 5-bromouracil and EMS, indicating that it may produce G • C or A • T base pairs. Some of these mutations are reversed by hydroxylamine, which produces only C • G → T • A transitions, indicating that some of the mutations produced by compound 2 generate C • G base pairs. None of the mutations are reversed by acridine orange, so compound 2 does not induce insertions or deletions. In summary, compound 2 produces single-base substitutions that generate both G • C and A • T base pairs.
c. Compound 3 produces mutations that are reversed only by acridine orange, so compound 3 appears to produce only insertions and deletions.
d. Compound 4 produces mutations that are reversed by 5-bromouracil, EMS, hydroxylamine, and acridine orange, indicating that this compound produces single-base substitutions that include the generation of G • C and A • T base pairs, as well as insertions and deletions.

Problem 2

Certain repeated sequences in eukaryotes are flanked by short direct repeats, suggesting that they originated as transposable elements. These same sequences lack introns and possess a string of thymine nucleotides at one end. Have these elements transposed as DNA or through an RNA intermediate? Explain your reasoning.

›› Solution Strategy

What information is required in your answer to the problem?

Whether the transposable elements transposed as DNA or through an RNA intermediate, and why you drew this conclusion.

What information is provided to solve the problem?

- The elements are flanked by short direct repeats.
- The elements lack introns, and each has a string of Ts at one end.

For help with this problem, review:

The Process of Transposition in Section 18.4.

» Solution Steps

The absence of introns and the string of thymine nucleotides (which would be complementary to adenine nucleotides in RNA) at one end are characteristics of processed RNA. These similarities to RNA suggest that the elements were originally transcribed into mRNA, processed to remove the introns and add a poly(A) tail, and then reverse transcribed into complementary DNA that was inserted into the chromosome.

Recall: Pre-mRNAs in eukaryotes are processed: a $5'$ cap is added, introns are removed, and a $3'$ poly(A) tail is added.

IMPORTANT TERMS

mutation
somatic mutation
germ-line mutation
gene mutation
base substitution
transition
transversion
insertion
deletion
frameshift mutation
in-frame insertion
in-frame deletion
expanding nucleotide repeat
forward mutation
reverse mutation (reversion)
missense mutation
nonsense mutation
silent mutation

neutral mutation
loss-of-function mutation
gain-of-function mutation
conditional mutation
lethal mutation
suppressor mutation
intragenic suppressor mutation
intergenic suppressor mutation
mutation rate
adaptive mutation
spontaneous mutation
induced mutation
incorporated error
replicated error
strand slippage
depurination
deamination
mutagen
base analog
intercalating agent
pyrimidine dimer
SOS system
carcinogen
Ames test
transposable element
flanking direct repeat
terminal inverted repeat
transposition
transposase
DNA transposon
retrotransposon
replicative transposition
nonreplicative transposition
insertion sequence (IS)
composite transposon
noncomposite transposon

hybrid dysgenesis
direct repair
base-excision repair
nucleotide-excision repair

ANSWERS TO CONCEPT CHECKS

1. c
2. A reverse mutation restores the original phenotype by changing the DNA sequence back to the wild-type sequence. A suppressor mutation restores the phenotype by causing an additional change in the DNA at a site that is different from that of the original mutation.
3. The factors that affect mutation rates are the frequency with which changes arise in DNA, how often these changes are repaired by DNA-repair mechanisms, and our ability to detect the mutation.
4. c
5. In transposition, staggered cuts are made in DNA at the point of insertion, leaving short single-stranded pieces of DNA on either side of the transposable element. Later, replication of these pieces creates flanking direct repeats on either side of the inserted transposable element.
6. Transposition often results in mutations because the transposable element inserts itself into a gene, destroying its function. Chromosome rearrangements arise because transposition includes the breaking and exchange of DNA sequences. Additionally, multiple copies of a transposable element may undergo homologous recombination, producing chromosome rearrangements.
7. d
8. a
9. d
10. Direct-repair mechanisms return an altered base to its correct structure without removing and replacing nucleotides. Mismatch repair and base-excision repair remove and replace nucleotides.
11. Changes in DNA structure may not undergo repair in people with defects in DNA-repair mechanisms. Consequently, increased numbers of mutations occur at all their genes, including mutations that predispose them to cancer. This observation indicates that cancer arises from mutations in DNA.

Chapter 18 Assessment

COMPREHENSION QUESTIONS

Section 18.1

1. What is the difference between a transition and a transversion? Which type of base substitution is more common?
2. Briefly describe expanding nucleotide repeats. How do they account for the phenomenon of anticipation?
3. What is the difference between a missense mutation and a nonsense mutation? Between a silent mutation and a neutral mutation?
4. Briefly describe two different ways in which intragenic suppressors can reverse the effects of mutations.

Section 18.2

5. How do insertions and deletions arise?
6. How do base analogs lead to mutations?
7. How do alkylating agents, nitrous acid, and hydroxylamine produce mutations?

Section 18.3

8. What is the purpose of the Ames test? How are *his*$^-$ bacteria used in this test?

Section 18.4

9. What general characteristics are found in many transposable elements?
10. How does a retrotransposon move?

11. Draw the structure of a typical insertion sequence, and identify its parts.
12. Explain how *Ac* and *Ds* elements produce variegated corn kernels.
13. What are some differences between Class I and Class II transposable elements?
14. Why are transposable elements often called genomic parasites?

Section 18.5

15. List at least three different types of DNA repair, and briefly explain how each is carried out.
16. What are the two major mechanisms for the repair of double-strand breaks? How do they differ?

APPLICATION QUESTIONS AND PROBLEMS

Section 18.1

17. CONNECT Mutations are often divided into gene mutations and chromosome mutations. This chapter discusses different types of gene mutations; chromosome mutations are discussed in Chapter 8. What are some types of chromosome mutations, and how do they differ from gene mutations?

18. CONNECT A codon that specifies the amino acid Gly undergoes a single-base substitution to become a nonsense mutation. In accord with the genetic code given in **Figure 15.10**, is this mutation a transition or a transversion? At which position of the codon does the mutation occur?

19. *Refer to the genetic code in **Figure 15.10** to answer the following questions:

a. If a single transition occurs in a codon that specifies Phe, what amino acids can be specified by the mutated sequence?
b. If a single transversion occurs in a codon that specifies Phe, what amino acids can be specified by the mutated sequence?
c. If a single transition occurs in a codon that specifies Leu, what amino acids can be specified by the mutated sequence?
d. If a single transversion occurs in a codon that specifies Leu, what amino acids can be specified by the mutated sequence?

20. Hemoglobin is a complex protein that contains four polypeptide chains. The normal hemoglobin found in adults—called adult hemoglobin—consists of two alpha and two beta polypeptide chains, which are encoded by different loci. Sickle-cell hemoglobin, which causes sickle-cell anemia, arises from a mutation in the beta chain of adult hemoglobin. Adult hemoglobin and sickle-cell hemoglobin differ in a single amino acid: the sixth amino acid from one end in adult hemoglobin is glutamic acid, whereas sickle-cell hemoglobin has valine at this position. After consulting the genetic code provided in **Figure 15.10**, indicate the type and location of the mutation that gave rise to sickle-cell anemia.

21. The following nucleotide sequence is found on the template strand of DNA. First, determine the amino acids of the protein encoded by this sequence by using the genetic code provided in **Figure 15.10**. Then give the altered amino acid sequence of the protein that will be found in each of the following mutations:

Description

The sequence reads, 3 prime T (nucleotide number 1) A C T G G C C G T T A G T T G A T A T A A C T (nucleotide number 24) 5 prime.

a. Mutant 1: A transition at nucleotide 11
b. Mutant 2: A transition at nucleotide 13
c. Mutant 3: A one-nucleotide deletion at nucleotide 7
d. Mutant 4: A T → A transversion at nucleotide 15
e. Mutant 5: An addition of TGG after nucleotide 6
f. Mutant 6: A transition at nucleotide 9

22. Draw a hairpin structure like that shown in **Figure 18.5** for the repeated sequence found in fragile-X syndrome (see **Table 18.1**).

23. *A polypeptide has the following amino acid sequence:

Met-Ser-Pro-Arg-Leu-Glu-Gly

The amino acid sequence of this polypeptide was determined in the series of mutants listed in parts *a* through *e*. For each mutant, indicate the type of mutation that occurred in the DNA (single-base substitution, insertion, deletion) and the phenotypic effect of the mutation (nonsense mutation, missense mutation, frameshift, etc.).

a. Mutant 1: Met-Ser-Ser-Arg-Leu-Glu-Gly
b. Mutant 2: Met-Ser-Pro
c. Mutant 3: Met-Ser-Pro-Asp-Trp-Arg-Asp-Lys
d. Mutant 4: Met-Ser-Pro-Glu-Gly
e. Mutant 5: Met-Ser-Pro-Arg-Leu-Leu-Glu-Gly

24. *A gene encodes a protein with the following amino acid sequence:

Met-Trp-His-Arg-Ala-Ser-Phe

A mutation occurs in the gene. The mutant protein has the following amino acid sequence:

Met-Trp-His-Ser-Ala-Ser-Phe

An intragenic suppressor mutation restores the amino acid sequence to that of the original protein:

Met-Trp-His-Arg-Ala-Ser-Phe

Give at least one example of base changes that could produce the original mutation and the intragenic suppressor. (Consult the genetic code in **Figure 15.10**.)

25. A gene encodes a protein with the following amino acid sequence:

Met-Lys-Ser-Pro-Ala-Thr-Pro

A nonsense mutation caused by a single-base-pair substitution occurs in this gene, resulting in a protein with the amino acid sequence Met-Lys. An intergenic suppressor mutation allows the gene to produce the full-length protein. With the original mutation and the intergenic suppressor present, the gene now produces a protein with the following amino acid sequence:

Met-Lys-Cys-Pro-Ala-Thr-Pro

Give the location and nature of the original mutation and of the intergenic suppressor.

Section 18.2

26. *Can nonsense mutations be reversed by hydroxylamine? Why or why not?

27. The following nucleotide sequence is found in a short stretch of DNA:

$$5'-\mathrm{ATGT}-3'$$
$$3'-\mathrm{TACA}-5'$$

If this sequence is treated with hydroxylamine, what sequences will result after replication?

28. *The following nucleotide sequence is found in a short stretch of DNA:

$$5'-\mathrm{GT}-3'$$
$$3'-\mathrm{TC}-5'$$

a. Give all the mutant sequences that can result from spontaneous depurination in this stretch of DNA.
b. Give all the mutant sequences that can result from spontaneous deamination in this stretch of DNA.

29. In many eukaryotic organisms, a significant proportion of cytosine bases are naturally methylated to 5-methylcytosine. Through evolutionary time, the proportion of AT base pairs in the DNA of these organisms increases. Can you suggest a possible mechanism for this increase?

Section 18.3

30. *A chemist synthesizes four new chemical compounds in the laboratory and names them PFI1, PFI2, PFI3, and PFI4. He gives the PFI compounds to a

geneticist friend and asks her to determine their mutagenic potential. The geneticist finds that all four are highly mutagenic. She also tests the capacity of mutations produced by the PFI compounds to be reversed by other known mutagens and obtains the following results. What conclusions can you make about the nature of the mutations produced by these compounds?

	Reversed by			
Mutations produced by	**2-Amino-purine**	**Nitrous acid**	**Hydroxyl-amine**	**Acridine orange**
PFI1	Yes	Yes	Some	No
PFI2	No	No	No	No
PFI3	Yes	Yes	No	No
PFI4	No	No	No	Yes

31. DATA ANALYSIS Mary Alexander studied the effects of radiation on mutation rates in the sperm of *Drosophila melanogaster*. She exposed *Drosophila* larvae to either 3000 roentgens (r) or 3975 r of radiation, collected the adult males that developed from the irradiated larvae, and mated them with nonirradiated females that were homozygous for recessive alleles at eight loci. She then counted the number of F_1 flies that carried a new mutation at each locus. All mutant flies that appeared were used in subsequent crosses to determine if their mutant phenotypes were heritable. For the *roughoid* locus, she obtained the following results (M. L. Alexander. 1954. *Genetics* 39:409–428):

Group	**Number of**	**Offspring with a mutation at the**

	offspring	*roughoid* locus
Control (0 r)	45,504	0
Irradiated (3000 r)	49,512	5
Irradiated (3975 r)	50,159	16

a. Calculate the mutation rates at the *roughoid* locus for the control group and the two groups of irradiated flies.
b. On the basis of these data, do you think radiation has any effect on mutation? Explain your answer.

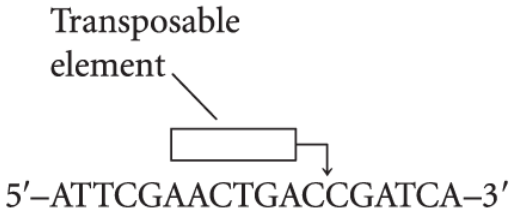
32. What conclusion would you draw if the numbers of bacterial colonies in Figure 18.23 were the same on the control plate and the treatment plate? Explain your reasoning.

33. A genetics instructor designs a laboratory experiment to study the effects of UV radiation on mutation in bacteria. In the experiment, the students spread bacteria on petri plates, expose the plates to UV light for different lengths of time, place the plates in an incubator for 48 hours, and then count the number of colonies that appear on each plate. The bacteria that have received more UV radiation should have more pyrimidine dimers, which block replication; thus, fewer colonies should appear on the plates exposed to UV light for longer periods. Before the students carry out the experiment, the instructor warns them that while the bacteria are in the incubator, the students must not open the incubator door unless the room is darkened. Why should the bacteria not be exposed to light?

Section 18.4

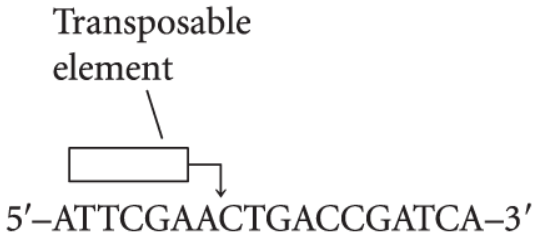
34. *A particular transposable element generates flanking direct repeats that are 4 bp long. Give the sequence that will be found on both sides of the transposable element if this transposable element inserts at the position indicated on each of the following sequences.

a.



Description
The sequence reads, 5 prime A T T C G A A C T G A C (Transposable element) C G A T C A 3 prime. A rectangular box represents transposable element and a downward arrow from it points to its location in the sequence.

b.



Description
The sequence reads, 5 prime A T T C G A A (transposable element) C T G A C C G A T C A 3 prime. A rectangular box represents transposable element and a downward arrow from it points to its location in the sequence.

35. CONNECT White eyes in *Drosophila melanogaster* result from an X-linked recessive mutation (see Chapter 4). Occasionally, white-eyed mutants give rise to offspring that possess white eyes with small red spots. The number, distribution, and size of the red spots are variable. Explain how a transposable element could be responsible for this spotting phenomenon.

36. What factor might potentially determine the length of the flanking direct repeats that are produced in transposition?

37. *Which of the following pairs of sequences might be found at the ends of an insertion sequence?
    a. $5'-\text{GGGCCAATT}-3'$ and $5'-\text{CCCGGTTAA}-3'$
    b. $5'-\text{AAACCCTTT}-3'$ and $5'-\text{AAAGGGTTT}-3'$
    c. $5'-\text{TTTCGAC}-3'$ and $5'-\text{CAGCTTT}-3'$
    d. $5'-\text{ACGTACG}-3'$ and $5'-\text{CGTACGT}-3'$
    e. $5'-\text{GCCCCAT}-3'$ and $5'-\text{GCCCAT}-3'$

38. Explain why the corn kernel in **Figure 18.34d** is variegated, with some areas colored and some areas lacking pigment.

39. *Two different strains of *D. melanogaster* are mated in reciprocal crosses. When strain A males are crossed with strain B females, the progeny are normal. However, when strain A females are crossed with strain B males, there are many mutations and chromosome rearrangements in the gametes of the F_1 progeny, and the F_1 generation is effectively sterile. Explain these results.

40. An insertion sequence contains a large deletion in its transposase gene. Under what circumstances would this insertion sequence be able to transpose?

41. CONNECT Zidovudine (AZT) is a drug used to treat patients with AIDS (see Chapter 9). AZT works by blocking the reverse-transcriptase enzyme used by the human immunodeficiency virus (HIV), the causative agent of AIDS. Do you expect that AZT would have any effect on transposable elements? If so, what type of transposable elements would be affected, and what would be the most likely effect?

42. A transposable element is found to encode a reverse transcriptase enzyme. On the basis of this information, what conclusions can you draw about the

likely structure and method of transposition of this element?

43. *A geneticist examines an ear of corn in which most kernels are yellow, but he finds a few kernels with purple spots, as shown here. Give a possible explanation for the appearance of the purple spots in these otherwise yellow kernels, accounting for the different sizes of the spots. (Hint: See the section on *Ac* and *Ds* elements in corn.)

Pierce, *Genetics: A Conceptual Approach*, 7e © 2020
W. H. Freeman and Company

Section 18.5

44. Which DNA-repair mechanism would most likely correct the incorporated error labeled by balloon 2 in **Figure 18.12**?

45. *A plant breeder wants to isolate mutants in tomatoes that are defective in DNA repair. However, this breeder does not have the expertise or equipment to study enzymes in DNA-repair systems. How can the breeder identify tomato plants that are deficient in DNA repair? What are the traits to look for?

CHALLENGE QUESTIONS

Section 18.1

46. DATA ANALYSIS Robert Bost and Richard Cribbs studied a strain of *E. coli* (*araB14*) that possessed a nonsense mutation in the structural gene that encodes L-ribulokinase, an enzyme that allows the bacteria to metabolize the sugar arabinose (R. Bost and R. Cribbs. 1969. *Genetics* 62:1–8). From the *araB14*

strain, they isolated some bacteria that possessed mutations that caused them to revert back to the wild type. Genetic analysis of these revertants showed that they possessed two different suppressor mutations. One suppressor mutation (*R1*) was linked to the original mutation in L-ribulokinase and probably occurred at the same locus. By itself, this mutation allowed the production of L-ribulokinase, but the enzyme produced was not as effective in metabolizing arabinose as was the enzyme encoded by the wild-type allele. The second suppressor mutation (Su^B) was not linked to the original mutation. In conjunction with the *R1* mutation, Su^B allowed the production of L-ribulokinase, but Su^B by itself was not able to suppress the original mutation.

a. On the basis of this information, are the *R1* and Su^B mutations intragenic suppressors or intergenic suppressors? Explain your reasoning.
b. Propose an explanation for how *R1* and Su^B restore the ability of *araB14* to metabolize arabinose and why Su^B is able to more fully restore this ability.

47. Achondroplasia is an autosomal dominant disorder characterized by disproportionate short stature: the legs and arms of people with achondroplasia are short compared with the head and trunk. The disorder is due to a base substitution in the gene, located on the short arm of chromosome 4, that encodes fibroblast growth factor receptor 3 (FGFR3).

AP Images/Gail Burton.

A family of three who have achondroplasia.

Although achondroplasia is clearly inherited as an autosomal dominant trait, more than 80% of the people who have achondroplasia are born to parents with normal stature. This high percentage indicates that most cases are caused by newly arising mutations; these cases (not inherited from an affected parent) are referred to as *sporadic*. Studies have demonstrated that sporadic cases of achondroplasia are almost always caused by mutations inherited from the father (paternal mutations). In addition, the occurrence of achondroplasia is higher among the children of older fathers; approximately 50% of children with achondroplasia are born to fathers older than 35 years of age. There is no association with maternal age. The mutation rate for achondroplasia (about 4×10^{-5} mutations per gamete) is high compared with that for other genetic disorders. Explain why most spontaneous mutations for achondroplasia are paternal in origin and why the occurrence of achondroplasia is higher among older fathers.

48. CONNECT Tay–Sachs disease (see p. 153 in Chapter 6) is a severe autosomal recessive genetic disease that produces deafness, blindness, seizures, and, eventually, death at 2 to 3 years of age. The disease results from a defect in the *HEXA* gene, which encodes hexosaminidase A. This enzyme normally degrades G_{M2} gangliosides. In the absence of hexosaminidase A, G_{M2} gangliosides accumulate in the brain. The results of molecular studies showed that the most common mutation causing Tay–Sachs disease is a 4-bp insertion that produces a downstream premature stop codon. Results of further studies have revealed that the transcription of the *HEXA* gene is normal in people who have Tay–Sachs disease, but the *HEXA* mRNA is unstable. Propose a mechanism to account for how a premature stop codon could cause mRNA instability.

49. DATA ANALYSIS *Ochre* and *amber* are two distinct nonsense mutations. Before the genetic code was worked out, Sydney Brenner, Anthony O. Stretton, and Samuel Kaplan applied different types of mutagens to bacteriophages in an attempt to determine the bases present in the codons responsible for *amber* and *ochre* mutations (S. Brenner et al. 1965. *Nature* 206:994–998). They knew that the *ochre* and *amber* mutations were suppressed by different types of suppressor mutations, which demonstrated that each is a different stop codon. They obtained the following results:

1. A single-base substitution could convert an *ochre* mutation into an *amber* mutation.
2. Hydroxylamine induced both *ochre* and *amber* mutations in wild-type phages.
3. 2-Aminopurine caused *ochre* to mutate to *amber*.
4. Hydroxylamine did not cause *ochre* to mutate to *amber*.

These data do not allow the complete nucleotide sequence of the *amber* and *ochre* codons to be worked out, but they do provide some information about the bases found in the nonsense mutations.

a. What conclusions about the bases found in the codons of *amber* and *ochre* mutations can be made from these observations?
b. Of the three nonsense codons (UAA, UAG, UGA), which represents the *ochre* mutation?

Section 18.4

50. DATA ANALYSIS Marilyn Houck and Margaret Kidwell proposed that *P* elements were carried from *Drosophila willistoni* to *D. melanogaster* by mites that feed on fruit flies (M. A. Houck et al. 1991. *Science* 253:1125–1129). What evidence do you think would be required to demonstrate that *D. melanogaster* acquired *P* elements in this way? Propose a series of experiments to provide such evidence.

Section 18.5

51. CONNECT Trichothiodystrophy is a human inherited disorder characterized by premature aging, including osteoporosis, osteosclerosis, early graying, infertility, and reduced life span. The results of studies showed that the mutation that causes this disorder occurs in a gene that encodes a DNA helicase (see Section 12.3). Propose a mechanism for how a mutation in a DNA helicase might cause premature aging. Be sure to relate the symptoms of the disorder to possible functions of the helicase enzyme.

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 18.1

1. Are mutations good or bad? Explain your response to this question.

2. Explain why loss-of-function mutations are frequently recessive, whereas gain-of-function mutations are frequently dominant.

3. As discussed, the overall rate of mutations in humans is estimated to be about 1×10^{-8} mutations per base pair per generation. How many new mutations would you expect each person to carry, on average, based on this mutation rate? Other studies have estimated that each person carries about 100 new loss-of-function mutations. How does this number compare with your estimate of the number of mutations based on the mutation rate? What might account for any differences?

Section 18.2

4. What accounts for the amazingly accurate replication of DNA, which keeps the mutation rate low?

Section 18.3

5. To determine whether radiation associated with the atomic bombings of Hiroshima and Nagasaki produced recessive germ-line mutations, scientists examined the sex ratio of the children of the survivors of the blasts. Can you explain why an increase in germ-line mutations might be expected to alter the sex ratio?

Section 18.4

6. Discuss the differences between transposable elements that are genomic parasites and transposable elements that have become domesticated. Do you think these terms (*genomic parasite* and *domesticated transposable elements*) are good choices for these types of elements? Why or why not?

Section 18.5

7. Research has shown that more mutations accumulate in regions of a chromosome that consist of compact chromatin, such as heterochromatin.

Offer an explanation for why mutation rates would be higher where chromatin is more compact.

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Create a concept map that outlines the relationship of expanding trinucleotide repeats to amyotrophic lateral sclerosis. Include at least the following terms: amyotrophic lateral sclerosis (ALS), degeneration of motor neurons, progressive weakness, sporadic ALS, inherited ALS, autosomal dominant trait, *C9orf72* gene, expanding nucleotide repeats, toxic dipeptides, frontotemporal dementia (FTD).

Section 18.1

2. Draw a concept map that summarizes the different types of mutations. Include the following concepts in your map: mutation, expanding nucleotide repeats, inherited change in DNA sequence, germ-line mutation, somatic mutation, base substitution, transversion, transition, indel, insertion, deletion, frameshift mutation, nonsense mutation, missense mutation, silent mutation, neutral mutation, loss-of-function mutation, gain-of-function mutation.

Section 18.4

3. Create a concept map that displays the characteristics and types of transposable elements and their functions by including least the following terms: transposable elements, DNA transposons, retrotransposons, flanking direct repeats, terminal inverted repeats, transposition, nonreplicative transposition, replicative transposition, cut-and-paste transposition, copy-

and-paste transposition, mutations, chromosome rearrangements, insertion sequences, composite transposons.

Section 18.5

4. Make a concept map that illustrates the major types of DNA repair and their features.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 19

Molecular Genetic Analysis and Biotechnology

Jaren Jai Wicklund/Shutterstock.

Duchenne muscular dystrophy is an X-linked recessive disorder. CRISPR-Cas9 genome editing techniques are being developed for possible use in treating children with this condition.

Editing the Genome with CRISPR-Cas9

One of the most remarkable of all DNA sequences is the human gene for dystrophin. This gene, located on the X chromosome, is

enormous, encompassing 2.2 million base pairs, including 79 exons and 78 introns. Its product, dystrophin, connects the cytoskeleton of a muscle cell to its extracellular matrix and is critical for muscle contraction.

Mutations in the dystrophin gene are the cause of Duchenne muscular dystrophy, a fatal muscle disorder that strikes nearly 1 in 3500 boys. At birth, these boys are healthy, but muscle weakness begins to appear between 3 and 5 years of age. As the disorder progresses, the child stumbles frequently, has difficulty climbing stairs, and is unable to rise from a sitting position. In time, the arm and leg muscles become progressively weaker. By age 11, most boys with the disorder must use a wheelchair, and by age 20, many have died. At present, there is no cure for the disease.

Duchenne muscular dystrophy was first recognized in 1852, and the disease was fully described in 1861 by Benjamin A. Duchenne, a French physician. Even before Mendel's laws were discovered, physicians noticed its X-linked pattern of inheritance, remarking that the disease developed almost exclusively in males and seemed to be inherited through unaffected mothers. In spite of this early recognition of its hereditary basis, the molecular basis of Duchenne muscular dystrophy remained a mystery for many years. In 1985, Louis Kunkel and his colleagues at Harvard Medical School observed that the X chromosome of a boy with Duchenne muscular dystrophy had a visible deletion. Reasoning that this boy's disease was caused by the absence of a gene within that deletion, they used

the deletion to pinpoint the location on the X chromosome of a gene that, when mutated or absent, caused Duchenne muscular dystrophy. Kunkel and his colleagues eventually located and cloned the piece of DNA responsible for the disease and discovered the dystrophin gene.

Ever since the discovery of the dystrophin gene, researchers and physicians have dreamed of treating Duchenne muscular dystrophy with gene therapy—restoring dystrophin production by inserting a corrected copy of the dystrophin gene into the patient's cells—but the large size of the dystrophin gene has made this approach challenging. Recently, however, geneticists have developed a novel and interesting approach to gene therapy that makes use of a new technique for editing the genome.

Many of the mutations that cause Duchenne muscular dystrophy consist of duplications or deletions that cause frameshift mutations, leading to premature stop codons that produce a truncated, nonfunctional dystrophin. The huge dystrophin protein consists of several domains. Some of these domains are not essential for muscle function. Researchers proposed that if the exons where mutations occurred could be removed, the premature stop codons would be eliminated. The resulting protein would be missing some amino acids, but a dystrophin that provided some muscle function might still be produced.

The trick to making this strategy work is the ability to selectively edit DNA sequences that control the splicing of pre-mRNA. Until recently, that wasn't possible. But within the past few years, a powerful new tool, called CRISPR-Cas9, has been developed that allows precision editing of the genome. In recent experiments, CRISPR-Cas9 was used to engineer changes in the genomes of mice, dogs, and humans that bring about exon skipping in the dystrophin gene, demonstrating the potential feasibility of this approach.

The geneticists used a mouse model of Duchenne muscular dystrophy called *mdx* mice. These mice possess a premature stop codon in exon 23 of the dystrophin gene and exhibit many of the symptoms of the human disorder. The CRISPR-Cas9 system consists of a protein called Cas9 that cuts DNA, along with two RNA molecules that can be genetically engineered to guide Cas9 to specific sequences—in this case, to the ends of exon 23. The geneticists loaded viruses with genetic material encoding these CRISPR-Cas9 components, then injected *mdx* mice with the viruses.

The results were remarkable. Delivered by the virus, CRISPR-Cas9 entered muscle cells and produced double-stranded cuts on either side of exon 23. Exon 23, along with the disease-causing premature stop codon it contained, was removed from the genomes of some cells. The result was synthesis of a partially functional dystrophin protein that enhanced muscle function in the mice. In 2018, researchers used this same approach to treat Duchenne muscular

dystrophy in beagles, removing an exon with a mutation, which then restored dystrophin production in the dogs' muscles. Researchers have also applied this method to cultured human stem cells from patients with muscular dystrophy and showed that the procedure restored dystrophin protein expression in derived heart cells (cardiomyocytes). This suggests that the procedure might also work in humans. Although much work needs to be done before these methods can be used to treat human patients, the results are encouraging and suggest that CRISPR-Cas9 might someday be used to restore muscle function to boys with Duchenne muscular dystrophy.

THINK-PAIR-SHARE

- Why does Duchenne muscular dystrophy affect only boys? Is it possible for it to occur in girls? If so, how might this take place?
- Becker muscular dystrophy (BMD) is another type of muscular dystrophy that results from mutations in the dystrophin gene, but its symptoms are less severe than those of Duchenne muscular dystrophy (DMD). About 60% of the mutations that cause both DMD and BMD are deletions. In BMD but not DMD, some multiple of three base pairs is usually deleted. Propose an explanation for why the symptoms of BMD are usually less severe than those seen in DMD, and how this difference might be related to the types of deletions.

The possibility of using CRISPR-Cas9 to genetically engineer a treatment for muscular dystrophy illustrates the power of molecular techniques for manipulating DNA sequences. This chapter introduces some of the techniques used in molecular genetic analysis. We begin by considering the challenges of working

at the molecular level. We then examine a number of methods used to analyze and alter DNA, placing emphasis on several transformative techniques that are used to cut, clone, amplify, and sequence DNA. Finally, we explore some of the applications of molecular genetic analysis.

19.1 Genetics Has Been Transformed by the Development of Molecular Techniques

A vast array of molecular methods is now available for probing the nature of hereditary information and revealing the details of genetic processes. These molecular techniques have drastically altered the way in which genes are studied. Previously, information about the structure and organization of genes was gained by examining their phenotypic effects, but molecular genetic analysis now enables scientists to directly read the nucleotide sequences themselves. This analysis has provided new information about the structure and function of genes and has altered many fundamental concepts of genetics. Our detailed understanding of genetic processes, such as replication, transcription, translation, RNA processing, and gene regulation, has been obtained through the use of molecular genetic techniques. These techniques are also used in many other fields, including biochemistry, microbiology, developmental biology, neurobiology, evolution, and ecology. Molecular genetic techniques are also being used to create a number of commercial products, including drugs, hormones, enzymes, and crops (**Figure 19.1**). The **biotechnology** industry has grown up around the use of these techniques to develop new products. In medicine, molecular genetic analysis is being used to probe the nature of cancer,

diagnose genetic and infectious diseases, produce drugs, and treat hereditary disorders.

Chris Knapton/Science Source.

19.1 Molecular genetic techniques have been used to create genetically modified crops. Genetically engineered corn now constitutes 92% of all corn grown in the United States.

Key Innovations in Molecular Genetics

The field of molecular genetics had its inception with the discovery of the three-dimensional structure of DNA by Watson, Crick, Franklin, and Wilkins in 1953. Since that discovery, the field has witnessed at least four major transformations, each driven by the development of powerful new techniques that provided important new insights into basic genetic processes. These seminal transformations include (1) the development of recombinant DNA technology, which allowed DNA from different sources to be

combined; (2) the invention of the polymerase chain reaction, which allowed very small quantities of specific DNA fragments to be quickly amplified; (3) the development of quick and accurate methods of determining DNA sequences; and (4) the engineering of CRISPR-Cas systems for accurate and efficient editing of genome sequences. In this chapter, we discuss each of these methods, along with supporting techniques and some of their applications.

THINK-PAIR-SHARE Question 1

CONCEPTS

Molecular genetic techniques are used to locate, analyze, alter, sequence, study, and recombine DNA sequences. These techniques are used to probe the structure and function of genes, address questions in many areas of biology, create commercial products, and diagnose and treat diseases. Four key innovations that have revolutionized genetics include recombinant DNA technology, the polymerase chain reaction, DNA sequencing technology, and genome editing methods.

Working at the Molecular Level

The manipulation of genes at the molecular level presents a serious challenge, often requiring strategies that may not seem obvious at first. The basic problem is that genes are minute, and every cell

contains thousands of them. Individual nucleotides cannot be seen, and no physical features mark the beginning or the end of a gene.

Let's consider a typical situation faced by a molecular geneticist. Suppose we want to use bacteria to produce large quantities of a human protein. The first and most formidable problem is finding the gene that encodes the desired protein. A haploid human genome consists of 3.2 billion base pairs of DNA. Let's assume that the gene that we want to isolate is 3000 bp long. Our target gene occupies only one-millionth of the genome, so searching for our gene in the huge expanse of genomic DNA is more difficult than looking for the proverbial needle in a haystack. But even if we are able to locate the gene, how do we separate it from the rest of the DNA?

If we succeed in locating and isolating the desired gene, we will next need to insert it into a bacterial cell. Linear fragments of DNA are quickly degraded by bacteria, so the gene must be inserted in a stable form. It must also be able to replicate successfully, or it will not be passed on when the cell divides. If we succeed in transferring our gene to bacteria in a stable form, we must still ensure that the gene is properly transcribed and translated. Furthermore, the methods used to isolate and transfer genes are inefficient: of a million bacterial cells that are subjected to these procedures, only *one* cell might successfully take up and express the human gene. So we must search through many cells to find the

one containing the recombinant DNA. We are back to the problem of the needle in a haystack.

Although these problems might seem insurmountable, molecular techniques have been developed to overcome them. Human genes are now routinely transferred to, and expressed in, bacterial cells.

CONCEPTS

Molecular genetic analyses require special techniques because individual genes make up a tiny fraction of the cellular DNA and cannot be seen.

19.2 Molecular Techniques Are Used to Cut and Visualize DNA Sequences

Often a first step in the molecular analysis of a DNA segment or gene is to isolate it from the remainder of the DNA so that further analyses can be carried out. In the sections that follow, we define recombinant DNA technology and consider three groups of molecular techniques that are used to cut DNA segments: restriction enzymes, engineered nucleases, and CRISPR-Cas systems.

Recombinant DNA Technology

Techniques for accurately and efficiently cleaving DNA helped to usher in the first major revolution in molecular genetics: the development of recombinant DNA technology. In 1973, a group of scientists produced the first organisms with recombinant DNA molecules. The group was led by Stanley Cohen of Stanford University and Herbert Boyer of the San Francisco School of Medicine at the University of California. The scientists inserted a piece of DNA from one plasmid into another, creating an entirely new recombinant DNA molecule. They then introduced the recombinant plasmid into *Escherichia coli* cells. These experiments ushered in one of the most momentous revolutions in the history of science.

Recombinant DNA technology is a set of molecular techniques for locating, isolating, altering, and studying DNA segments. The term *recombinant* is used because frequently, the goal is to combine DNA from two distinct sources. Genes from two different bacteria might be joined, for example, or a human gene might be inserted into a viral chromosome. Commonly called **genetic engineering**, recombinant DNA technology now encompasses many molecular techniques that can be used to analyze, alter, and recombine virtually any DNA sequences from any number of sources.

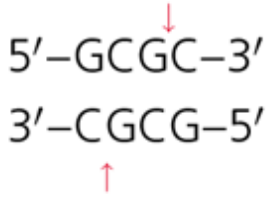
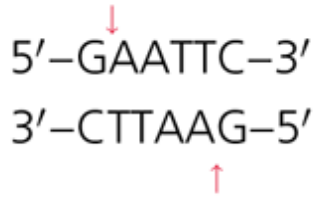
Restriction Enzymes

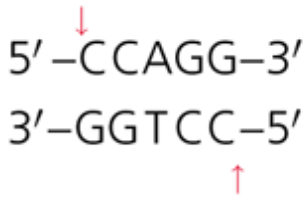
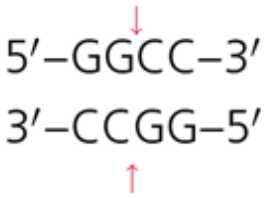
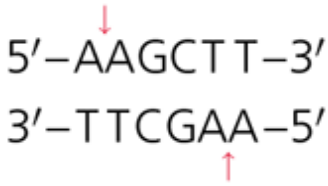
A key event in the development of recombinant DNA technology was the discovery, in the late 1960s, of **restriction endonucleases** (also commonly called **restriction enzymes**), which recognize specific nucleotide sequences in DNA and make double-stranded cuts at those sequences (called *restriction sites*). Restriction enzymes are produced naturally by bacteria and are used in defense against viruses. A bacterium protects its own DNA from a restriction enzyme by modifying the recognition sequence, usually by adding methyl groups to its DNA.

More than 800 different restriction enzymes that recognize and cut DNA at more than 100 different sequences have been isolated from bacteria. Many of these enzymes are commercially available; examples of some commonly used restriction enzymes are given in

Table 19.1. The name of each restriction enzyme begins with an abbreviation that signifies its bacterial origin.

TABLE 19.1 Characteristics of some common restriction enzymes used in recombinant DNA technology

Enzyme	Microorganism from Which Enzyme Is Produced	Recognition Sequence	Type of Fragment End Produced
*Bam*HI	*Bacillus amyloliquefaciens*		Cohesive
*Cof*I	*Clostridium formicoaceticum*		Cohesive
*Eco*RI	*Escherichia coli*		Cohesive
*Eco*RII	*Escherichia coli*		Cohesive

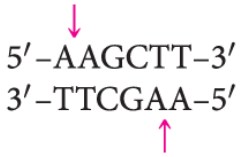
			
*Hae*III	*Haemophilus aegyptius*		Blunt
			
*Hin*dIII	*Haemophilus influenza*		Cohesive
			
*Pvu*II	*Proteus vulgaris*		Blunt

Note: The first three letters of the abbreviation for each restriction enzyme refer to the bacterial species from which the enzyme was isolated (e.g., *Eco* refers to *E. coli*). A fourth letter may refer to the strain of bacteria from which the enzyme was isolated (the "R" in *Eco*RI indicates that this enzyme was isolated from the RY13 strain of *E. coli*).

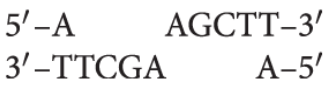
The sequences recognized by restriction enzymes are usually 4–8 bp long; most of these enzymes recognize a sequence of 4 or 6 bp. Most

recognition sequences are palindromic—sequences that read the same (5′ to 3′) on the two complementary DNA strands.

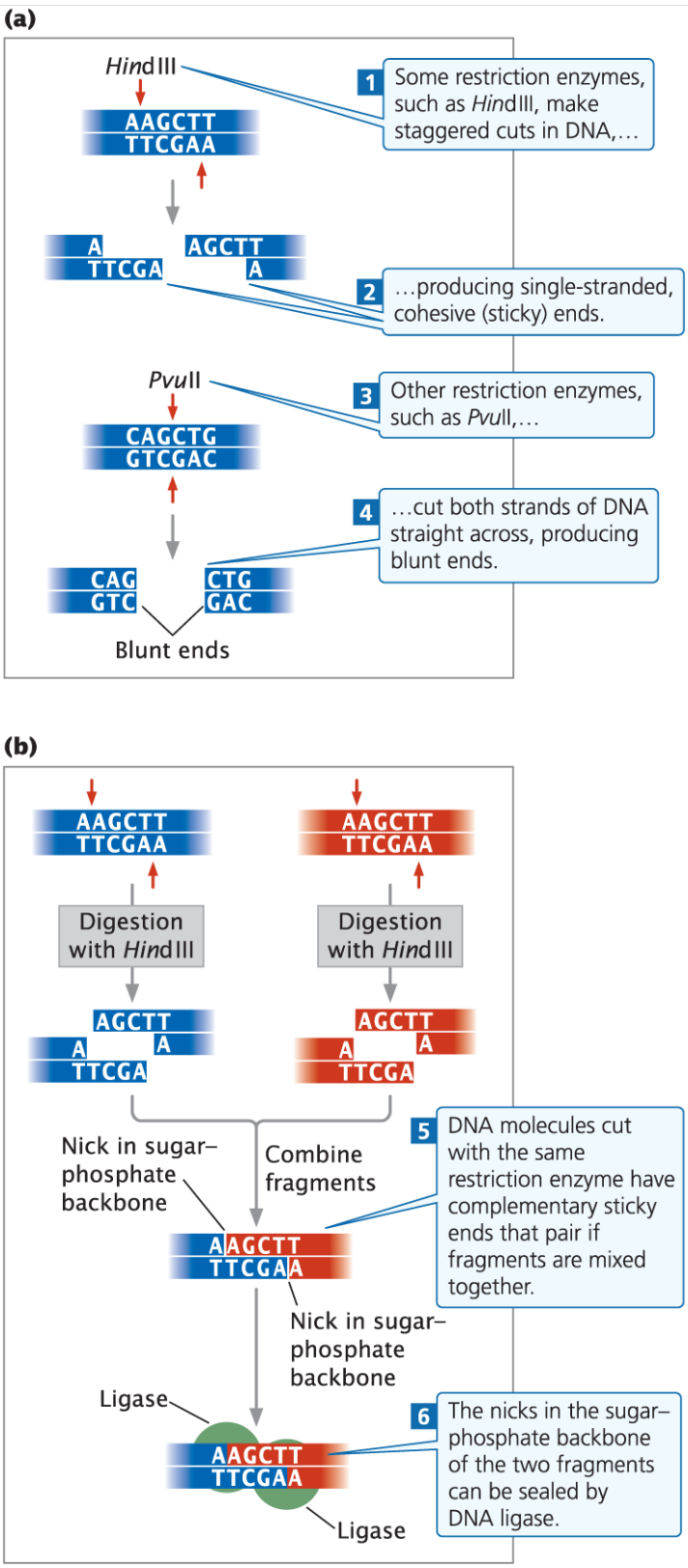
Some restriction enzymes make staggered cuts in the DNA. For example, *Hin*dIII recognizes the following sequence:



*Hin*dIII cuts the sugar–phosphate backbone of each strand at the point indicated by the arrow, generating fragments with short single-stranded overhanging ends:



Such ends are called **cohesive ends**, or *sticky ends*, because they are complementary to each other and can spontaneously pair to connect the fragments. Thus, DNA fragments with sticky ends can be "glued" together: any two such fragments cleaved by the same enzyme will have complementary ends and will pair (**Figure 19.2**). When their cohesive ends have paired, the two DNA fragments can be joined together permanently by DNA ligase, which seals nicks between the sugar–phosphate groups of the fragments.



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020
W. H. Freeman and Company

19.2 Restriction enzymes make double-stranded cuts in DNA, producing cohesive, or sticky, ends.

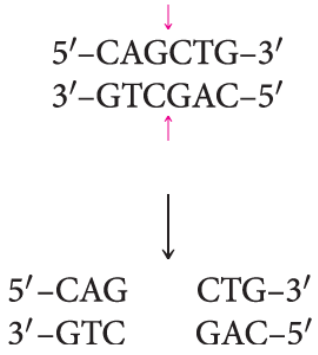
Description

Part “a” shows sticky ends produced by Hind 3 and blunt ends produced by P V U 2. Hind 3 acts on the D N A sequence as follows: strand 1-A (an arrow points between) A G C T T, strand 2-T T C G A (an arrow points between) A. This leads to a D N A sequence having a nick in the areas where arrows were present. A caption reads, “Some restriction enzymes, such as Hind 3, make staggered cuts in D N A, producing single-stranded, cohesive (sticky) ends.” P V U 2 acts on a D N A sequence as follows: strand 1-C A G (an arrow points between) C T G, strand 2-G T C (an arrow points between) G A C. This leads to a D N A sequence having a nick in the areas where arrows were present. The D N A fragments are labeled blunt ends. A caption reads, “Other restriction enzymes, such as P V U 2 cut both strands of D N A straight across, producing blunt ends.”

Part “b” shows two different D N A molecules cut with the same restriction enzyme, Hind 3. A flowchart is shown. Initially, the flowchart has two branches having the same D N A sequence in two different colors. First branch shows the D N A sequence as follows: strand 1-A (an arrow points between) A G C T T, strand 2-T T C G A (an arrow points between) A in blue color. Second branch shows the same sequence in red. Both the D N A sequences are digested with Hind 3 producing nicks where arrows were present. A downward arrow then merges the two branches and is labeled “combine fragments”. A D N A sequence is then shown as follows: strand 1-A (blue) A G C T T (red), strand 2-T T C G A (blue) A (red). The places in the sequence where red and blue color merge are labeled nick in sugar-phosphate backbone. A caption reads, “D N A molecules cut with the same restriction enzyme have complementary sticky ends that pair if fragments are mixed together.” A downward arrow leads to the same D N A sequence having ligase bound to the places where nicks were present. A caption reads, “The nicks in the sugar–phosphate backbone of the two fragments can be sealed by D N A ligase.”

Not all restriction enzymes produce staggered cuts and sticky ends (see Figure 19.2a). *Pvu*II cuts in the middle of its recognition sequence, and the cuts on the two strands are directly opposite each

other, producing blunt-ended fragments that must be joined together in other ways:



Description
The template D N A strand has the following sequence: 5 prime-C A G C T G-3 prime, 3 prime-G T C G A C-5 prime. A downward arrow leads to blunt ends produced as follows: 5 prime- C A G nick C T G-3 prime, 3 prime-G T C nick G A C-5 prime.

The sequences recognized by a restriction enzyme are located randomly within the genome. Accordingly, there is a relation between the length of the recognition sequence and the number of times it is present in a genome: there will be fewer longer recognition sequences than shorter ones, because the probability of the occurrence of a particular sequence consisting of, say, six specific bases is less than the probability of the occurrence of a particular sequence of four specific bases. Therefore, restriction enzymes that recognize longer sequences will cut a given piece of DNA into fewer and longer fragments than will restriction enzymes that recognize shorter sequences.

Restriction enzymes are used whenever DNA fragments must be cut or joined. In a typical restriction reaction, a concentrated solution of purified DNA is placed in a small tube with a buffer solution and a small amount of restriction enzyme. The reaction mixture is then heated to the optimal temperature for the enzyme, usually 37°C. Often within an hour, the enzyme cuts the appropriate restriction sites in all the DNA molecules, producing a set of DNA fragments. ›

TRY PROBLEM 34

CONCEPTS

Restriction enzymes cut DNA at specific base sequences that are palindromic. Some restriction enzymes make staggered cuts, producing DNA fragments with cohesive ends; others cut both strands straight across, producing blunt-ended fragments. There are fewer long recognition sequences in DNA than there are short recognition sequences.

✔ CONCEPT CHECK 1

Where do restriction enzymes come from?

Engineered Nucleases

One limitation of restriction enzymes is that because their recognition sequences are short — typically 4–8 bp in length — they occur at random many times within a genome. For example, consider the 6-bp recognition sequence for *Bam*HI:

5′–GGATCC–3′
3′–CCTAGG–5′

There are over 47,000 *Bam*HI restriction sites in the human genome. Cutting human DNA with *Bam*HI or another restriction enzyme results in thousands of fragments. Therefore, it is impossible to precisely cut genomic DNA at a single location with restriction enzymes.

To overcome this problem of numerous cuts, geneticists have sought nucleases that recognize longer DNA sequences. In recent years, geneticists have designed complex enzymes, termed **engineered nucleases**, that are capable of making unique double-stranded cuts in DNA at predetermined sequences. Engineered nucleases consist of the part of a restriction enzyme that cleaves DNA nonspecifically, coupled with another protein that recognizes and binds to a specific DNA sequence; the particular sequence to which the protein binds is determined by the protein's amino acid sequence. By altering the amino acid sequence of the binding protein, geneticists can custom-design a nuclease to bind to, and cut, any particular DNA sequence.

Engineered nucleases include **zinc-finger nucleases (ZFNs)**, which use a DNA-binding domain called a zinc finger (see Section 16.1) attached to a restriction enzyme, most often *Fok*I. In these nucleases, several zinc-finger domains are combined in an array; the most commonly used ZFNs have three of these domains, which together

recognize a 9-bp DNA sequence. To make double-stranded cuts in DNA, ZFNs are used in pairs, which increases their specificity to 18 or more base pairs—enough to ensure that most genomes are cut only once. Another type of engineered nuclease is a **transcription activator–like effector nuclease (TALEN)**, in which a protein that normally binds to sequences in promoters is attached to the *Fok*I restriction enzyme. The binding protein consists of a series of repeats whose amino acid sequence determines the DNA base sequence that it recognizes.

The key to both ZFNs and TALENs is the ability to alter the amino acid sequence of the binding proteins in these nucleases such that they bind to a specific target DNA sequence. For ZFNs, there is no simple correspondence between the amino acid sequence of the binding protein and the DNA sequence to which it binds, so each ZFN must be custom-designed for a particular sequence. For TALENs, a more straightforward relation exists between the amino acid sequence of the binding protein and the base sequence of the target DNA, but making specific proteins for individual DNA sequences is laborious and costly.

CRISPR-Cas Genome Editing

Another molecular tool for precisely cutting DNA is the **CRISPR-Cas system**. This technique has revolutionized the field of genetics, providing a powerful way of editing the genome that has now been

applied to DNA sequences in bacteria, yeast, nematodes, plants, fruit flies, zebrafish, mice, rats, monkeys, humans, and many more organisms.

CRISPR-Cas IMMUNITY IN BACTERIA AND ARCHAEA

CRISPR-Cas systems occur naturally in bacteria and archaea and are used to protect these organisms against bacteriophages, plasmids, and other invading DNA elements (see Chapters 9 and 14). CRISPR RNAs (crRNAs) are encoded by DNA sequences called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). A CRISPR array consists of a series of such palindromic sequences (sequences that read the same forward and backward on two complementary DNA strands) separated by unique spacers, which consist of sequences derived from bacteriophages or foreign plasmids (see Figure 14.23a). When bacteriophage or plasmid DNA enters a prokaryotic cell, proteins cut up the foreign DNA and insert bits of it into a CRISPR array, which then serves as a memory of the invader. The DNA sequences in bacteriophage or plasmid DNA that match the spacer elements in the CRISPR array are referred to as *protospacers*.

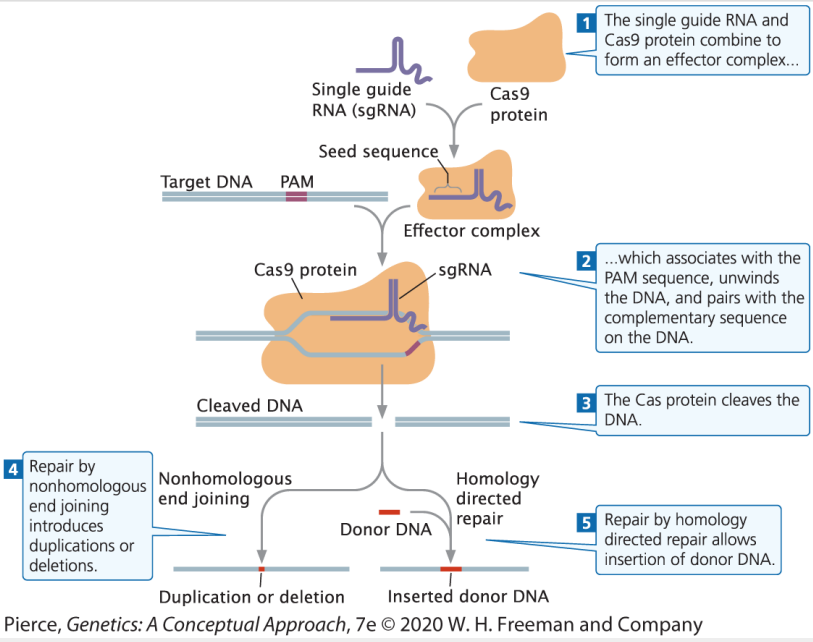
The CRISPR array is transcribed into a long precursor CRISPR RNA (pre-crRNA), which is cleaved into short crRNAs. The crRNAs combine with proteins called CRISPR-associated (Cas) proteins to form effector complexes. The Cas proteins have nuclease activity—

the ability to cut DNA. If the same foreign DNA (the protospacer) enters the cell in the future, a CRISPR-Cas complex recognizes and attaches to it. The crRNA in the complex binds to its complementary sequence in the foreign DNA, and the Cas protein cleaves the foreign DNA, rendering it nonfunctional. In this way, CRISPR-Cas serves as an adaptive RNA defense system that remembers and destroys foreign invaders.

GENOME EDITING WITH CRISPR-Cas

A variety of CRISPR-Cas systems have been found in different bacterial and archaeal species. Geneticists have engineered some of these systems to serve as molecular editing tools. The most widely used system is CRISPR-Cas9, derived from the bacterium *Streptococcus pyogenes*. This system naturally requires two RNA molecules, crRNA and another RNA molecule termed tracrRNA. These two RNAs pair and then combine with Cas9 to form an effector complex. To facilitate the use of this system in genome editing, researchers have engineered the crRNA and the tracrRNA into a single guide RNA (sgRNA) (**Figure 19.3**). A 20-nucleotide region of the sgRNA pairs with DNA, although the nucleotides within an 8–12-nucleotide "seed" sequence are most important in pairing. By altering the sequence of the sgRNA, it is possible to direct the action of the effector complex to any specific DNA sequence desired. This relatively long recognition sequence makes CRISPR-Cas9 much more

specific than restriction enzymes, meaning that it can be directed to unique sites within the genome.



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19.3 CRISPR-Cas9 is a technique for precisely editing the genome.

Description

An R N A molecule containing a stem, also called single-guide R N A, binds to Cas 9 protein. A caption reads, “The single guide R N A and Cas 9 protein combine to form an effector complex which associates with the PAM sequence, unwinds the D N A, and pairs with the complementary sequence on the D N A.” The complex formed is called the effector complex. The left part of the single-guide R N A in the effector complex has a seed sequence. The effector complex binds to a target D N A strand near the PAM sequence, unwinds part of the D N A forming a bubble at the center, and cleaves the D N A into 2 double-stranded molecules. A caption reads, “The Cas protein cleaves the D N A.” The strands are repaired by either nonhomologous end joining, which can introduce duplications or deletions, or homologous recombination. In homology directed repair, a donor D N A molecule is inserted between the breaks. A caption reads, “Repair by nonhomologous end joining introduces duplications or deletions.” Another caption reads, “Repair by homology directed repair allows insertion of donor D N A.”

An important feature of the CRISPR-Cas9 system is the required presence of a sequence in the target DNA (the DNA to be cleaved) called a protospacer-adjacent motif (PAM; see Figure 19.3). These sequences are short and have a weak consensus (the PAM for the *S. pyogenes* system is 5′–NGG–3′, where N represents any base and G represents guanine), so they occur at numerous random places throughout most genomes. For example, the CRISPR-Cas9 PAM is estimated to occur, on average, about once every 8 bp in the human genome. The CRISPR-Cas9 effector complex first associates with a PAM, and then Cas9 unwinds the DNA nearby. This unwinding allows the sgRNA to pair with its complementary sequence in the DNA. Once the target DNA and sgRNA are paired, Cas9 makes double-stranded cuts in the DNA.

REPAIR OF BREAKS PRODUCED BY CRISPR-Cas9

Once the DNA of a cell has been cleaved by CRISPR-Cas9, the cell immediately uses its DNA-repair mechanisms to try to repair the break. This feature provides a mechanism for editing the target sequence. There are two major pathways by which double-strand breaks are repaired within cells (see Section 18.5). One, called nonhomologous end joining, joins together the two ends of DNA without using any template. The CRISPR-Cas9 system can be targeted to a specific gene; when that gene is cleaved by Cas9 and then repaired by nonhomologous end joining small insertions and deletions are introduced at the break site. This often produces

frameshift mutations that disrupt the coding sequence and disable the gene (see Figure 19.3).

The other mechanism used by cells to repair double-strand breaks is homology directed repair (see Section 18.5). This mechanism functions when a DNA template is provided for repairing the break. A researcher can provide a donor piece of double-stranded DNA that has ends complementary to the sequences at the ends of the break made by Cas9; homology directed repair may insert the donor DNA sequence into the break. In this way, researchers can selectively insert a desired sequence into a genome (see Figure 19.3). Unfortunately, homology directed repair is not highly efficient, and often the two ends are connected without insertion of the donor DNA.

THINK-PAIR-SHARE Question 2

ADVANTAGES OF CRISPR-Cas9

An advantage of CRISPR-Cas9 over restriction enzymes is the relatively long nucleotide sequence recognized by the sgRNA, which allows researchers to produce unique cuts within genomic DNA. By changing the sequence of the sgRNA, precise edits can be made almost anywhere in the genome. Modifying the sgRNA to match a particular sequence in the genome is much simpler than trying to alter a DNA-binding protein, such as the proteins in zinc-finger

nucleases and TALENs. Another important feature of CRISPR-Cas9 is the ability to use this technology in intact cells; mRNA for the Cas9 protein and specific sgRNAs can be introduced into many types of cells, where they are expressed and carry out their editing function. In a process known as multiplexing, it is possible to introduce several sgRNAs into a cell simultaneously and carry out multiple cuts in a single step.

CRISPR-Cas has great potential for genetic engineering and biotechnology. It can be applied to many different species, including species in which other methods of DNA manipulation have not worked well. It can be used to introduce new DNA sequences into whole animals and humans. For example, it is already being used to induce specific mutations in mice to create genetic models of human diseases, which can then serve as powerful research tools for the study of those diseases. CRISPR-Cas is also being developed as a tool for correcting genetic defects (gene therapy) and has the potential for treating infectious diseases by eliminating viral DNA from human cells. It will enable genetic modification of crops and domestic animals, in which it can be used to create very specific genetic alterations that benefit yield and produce characteristics that improve cultivation.

MODIFICATIONS OF CRISPR-Cas

Researchers have developed a number of modifications of CRISPR-Cas systems to provide additional functions. For example, the Cas9

protein can be modified so that it makes single-stranded cuts in DNA, which are more likely to be repaired by homology directed repair and other precise DNA-repair mechanisms. Nucleases of other CRISPR systems are being used for different types of cutting. A Cas protein called Cpf1, which is used in some CRISPR systems, makes staggered cuts that produce complementary sticky ends like those produced by some restriction enzymes. The production of complementary sticky ends makes repair more accurate and enables the insertion of desired sequences with complementary sticky ends. Other CRISPR-Cas systems, called base editors, have been engineered to make single pair base substitutions in DNA and RNA, allowing precise changes to nucleotide sequences.

When a PAM for Cas9 is not available near a site where cleavage is desired, Cas proteins from different species that recognize different PAMs can be used. Variants of Cas9 have also been engineered that use different or more common PAM sequences. For example, one engineered variant of Cas9 recognizes the PAM sequence NG, which occurs more commonly than the usual NGG PAM recognized by Cas9.

Modified CRISPR-Cas systems (with their cleavage function inactivated) have been developed for use as general RNA-guided devices for other functions. Transcription factors or chromatin-remodeling proteins can be tethered to an inactivated Cas to affect transcription at specific sites in the genome. For example, CRISPR-Cas has been modified to inhibit gene expression. Called CRISPR-

mediated interference (CRISPRi), this system combines an inactive form of Cas protein fused to a Krüppel-associated box (KRAB) protein domain (a zinc-finger binding domain; see Table 16.1), which inhibits transcription by producing chromatin modifications around transcription start sites. The sgRNA of CRISPRi targets this complex to a specific gene promoter, and the KRAB domain then inhibits the transcription of the gene. CRISPRi has been used to test the functional significance of long noncoding RNAs (lncRNAs; see Chapter 14). Researchers found that when expression of many of the lncRNAs was inhibited, cell growth was negatively affected, suggesting that the lncRNAs have important functions. Inactivated Cas proteins have also been coupled with proteins that stimulate transcription.

In another application, a CRISPR system called EvolvR targets an error-prone DNA polymerase to specific sites, where it synthesizes DNA with a high frequency of mutations, enabling the experimental study of evolution. CRISPR-Cas has also been adapted for use as a quick and efficient diagnostic tool for detecting viruses. For this function, researchers use a CRISPR-Cas system that attaches to specific viral DNA or RNA and cleaves it. The cleavage by this particular Cas protein then unleashes indiscriminate cleavage of nearby nonspecific single-stranded DNA that has been added and to which a fluorescent or other color tag has been attached. This produces a visual signal that the viral DNA is present. The components of this system have been applied to a strip of paper, which changes color when the viral DNA is detected, creating a

diagnostic kit that provides a sensitive, rapid, and instrument-free method of detecting pathogens, all desirable properties for detecting viruses in remote areas. This method has been used in the detection of Zika virus, Dengue virus, and human papillomavirus (see Chapter 9).

In another application, researchers have developed a system they call CRISPR-mediated analog multi-event recording apparatus (CAMERA), which uses CRISPR to create alterations in cellular DNA when a specific event occurs. The alterations can be used as a record of the events, such as changes in gene expression, alterations in environmental conditions, or development alterations leading to a specific cell's lineages. These are just a few examples of the many ways that CRISPR-Cas has been modified and extended to provide a wide array of new molecular tools (**Table 19.2**).

THINK-PAIR-SHARE Question 3

TABLE 19.2 Functions of CRISPR-Cas Systems

Name of System	Function
Traditional CRISPR-Cas9	Cuts double-stranded DNA at specific sequences
CRISPR-Cas9 Systems[1]	Cuts single-stranded DNA
CRISPR-Cas13a	Cuts single-stranded RNA
CRISPR-HypaCas9	Cuts double-stranded DNA with high accuracy, very little off-target cleavage

Base editors	Create single base pair substitutions
xCas9s	Recognizes more common PAM sequences
CRISPRa	Activates transcription
CRISPRi	Inhibits transcription
EvolvR	Induces multiple mutations within a short section of DNA
SHERLOCK	Detects pathogen DNA or RNA
CAMERA	Creates a retrievable record in DNA

[1]CRISPR-Cas9 systems from various species

LIMITATIONS AND CHALLENGES OF CRISPR-Cas9 EDITING

A limitation in the use of CRISPR-Cas9 for genome editing is the potential for off-target cleavage. Some mismatches between the sgRNA and the complementary DNA sequence are tolerated by the Cas9 protein, so the system may cleave DNA at sites other than the desired target sequence. Unfortunately, off-target cleavage depends on many factors and varies among cell types, so predicting where and when cleavage will occur has been difficult. Much work has concentrated on engineering the sgRNA and Cas9 protein to be more selective in pairing and cutting, so as to reduce undesirable off-target cuts. For example, geneticists have produced a high-fidelity Cas9 by altering its amino acid sequence so that nonspecific interaction between the Cas protein and the DNA backbone is weakened, thereby forcing the nuclease to depend more on base pairing between the sgRNA and the DNA and increasing its

specificity. These changes eliminated nearly all off-target cleavage by the high-fidelity Cas9.

If CRISPR-Cas is applied to multicellular embryos or whole organisms, there is also the potential to create genetic mosaics (see Section 6.2). Genome editing with CRISPR-Cas is not 100% efficient, meaning that DNA in some cells is edited and DNA in other cells is not. It can therefore produce tissues in which different cells have different DNA sequences. The effects of this mosaicism could vary tremendously, depending on what genes are edited, what tissues are involved, and how efficient the editing is.

Another potential difficulty is getting the CRISPR-Cas components into a cell. This can often be done with cells in culture by transfection, in which cells are bathed in a solution containing DNA or mRNA encoding the Cas protein and the sgRNA and treated to make their membranes more permeable to foreign DNA. While transfection works in cell culture, getting the CRISPR-Cas components into intact organisms is more challenging. Sometimes the sgRNA and Cas protein are directly injected into fertilized egg cells, but most cells are too small for direct injection. Cas9 is a relatively large protein that does not easily pass across the cell membrane. Some other CRISPR-associated nucleases are smaller and easier to get into cells; these smaller nucleases are being exploited for use in delivery. Researchers have also delivered Cas enzyme to brain cells by coating the enzyme onto gold nanoparticles, which were then injected into the brain.

CONCERNS ABOUT THE USE OF CRISPR-Cas9 EDITING

One concern about CRISPR-Cas9 technology is that its ease of use and potential to alter almost any DNA sequence might mean that it could be used to genetically modify humans in ethically questionable ways. Gene therapy—the genetic alteration of somatic cells to treat disease—has been carried out numerous times using non-CRISPR technology and is widely accepted by bioethicists (see Section 19.7). ethical concerns about CRISPR-Cas focus on the possibility of germ-line editing (in which genes in reproductive cells are altered) because the edited genes will be passed on to future generations, affecting the future gene pool of the species. In early 2015, a group of leading scientists and ethicists met in Napa, California, to discuss the scientific, legal, and ethical implications of genome editing using CRISPR-Cas technology. This group strongly discouraged all attempts at human germ-line modification until the implications of CRISPR-Cas editing could be discussed more widely by scientific and governmental organizations. The group encouraged transparent research, educational forums on the new technology, and the convening of representative developers and users to discuss its social, ethical, and scientific implications. Other groups have echoed many of these same recommendations. In spite of these concerns, the CRISPR-Cas system has been used to carry out genome editing in human embryos, including correcting a mutation that causes a heart condition, although in this case the treatment was carried out in vitro and the embryos were never allowed to complete

development. In 2018, a Chinese researcher shocked the world by reporting that he had carried out gene alteration on human embryos, which were then allowed to develop and were born. The gene that was allegedly altered provided immunity to HIV, a disease that is preventable by other means. This work has been uniformly condemned as unethical by researchers and ethicists around the world.

In addition to ethical considerations, there are safety concerns about applying CRISPR-Cas to humans. Off-target cuts could induce mutations that might lead to cancer or other medical problems. Some researchers have pointed to the potential dangers of using CRISPR-Cas to edit animals and plants that are released into the wild.

THINK-PAIR-SHARE Question 4

CONCEPTS

The development of CRISPR-Cas9 technology provides a powerful means of cutting and editing the genome. CRISPR-Cas9 combines a single guide RNA with a nuclease, which together attach to specific DNA sequences and make double-stranded cuts at specific locations. Repair of these cuts by nonhomologous end joining or homology directed repair provides the means to introduce alterations to the genome. This technology has been used in many organisms and cell types, and it has the potential for additional applications, but its use has raised a number of ethical concerns.

Separating and Viewing DNA Fragments

We have now discussed several techniques for precisely cutting a DNA molecule. After cutting, it is often necessary to separate the resulting fragments and to verify that the DNA was altered in the expected fashion. Gel electrophoresis provides a way to separate and visualize DNA fragments.

Electrophoresis is a standard technique for separating molecules on the basis of their size and electrical charge. There are a number of different types of electrophoresis; to separate DNA molecules, **gel electrophoresis** is used. The gel most often used is a porous gel made from agarose (a polysaccharide isolated from seaweed), which is melted in a buffer solution and poured into a plastic mold. As it cools, the agarose solidifies, making a gel that looks something like stiff gelatin.

Small wells are made at one end of the gel, solutions of DNA fragments are placed in the wells (**Figure 19.4a**), and an electrical current is passed through the gel. The DNA fragments migrate toward the positive end of the gel. During this migration, the porous gel acts as a sieve, separating the DNA fragments by size. Small DNA fragments migrate more rapidly than do large ones, so over 1–2 hours, the fragments separate on the basis of their size. Typically, DNA fragments of known length (size standards) are placed in one of

the wells. By comparing the migration distance of the unknown fragments with the distance traveled by the size standards, a researcher can determine the approximate size of the unknown fragments (**Figure 19.4b**).

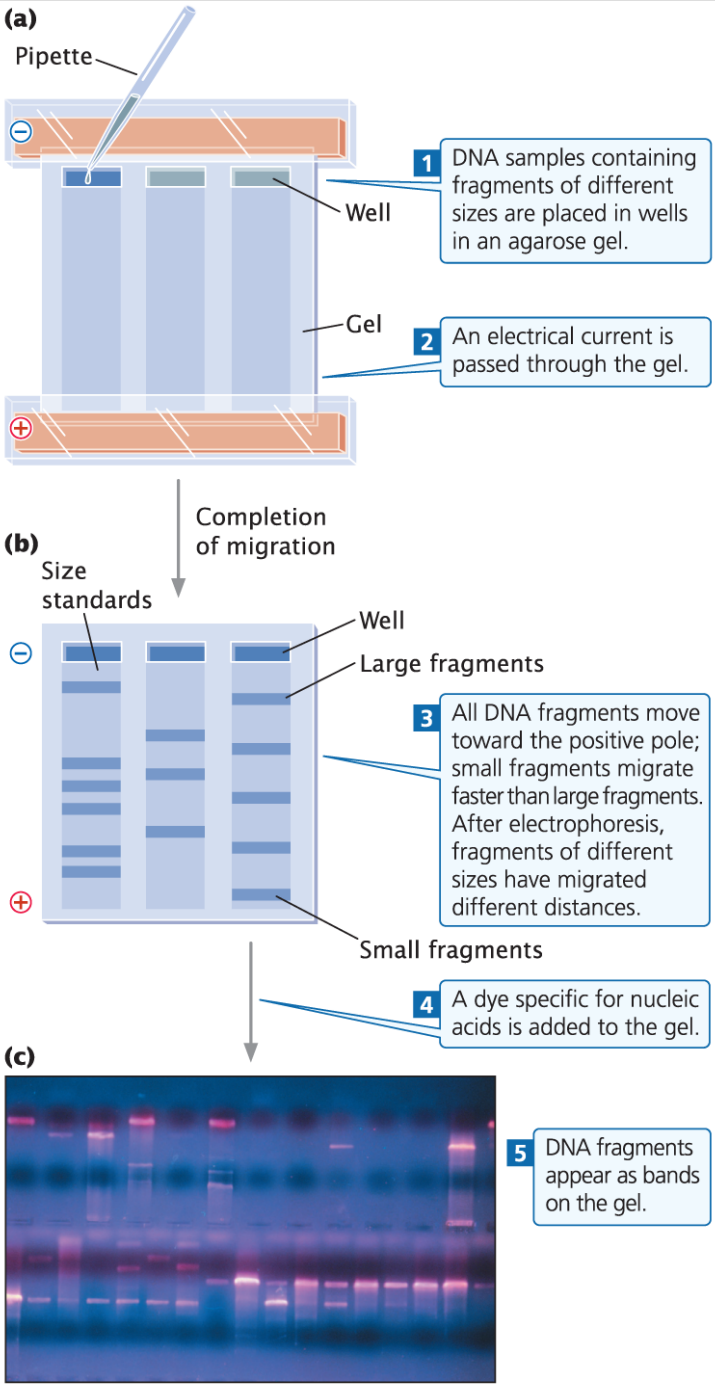

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Photo: Klaus Guldbrandsen/Science Source.

19.4 Gel electrophoresis can be used to separate DNA molecules on the basis of their size and electrical charge.

Description
Part “a” shows an electrophoretic chamber with wells and gel. D N A sample is being uploaded into a well through a pipette. The upper side of the chamber holds negative charge and the bottom holds positive charge. A caption reads, “D N A samples containing fragments of different sizes are placed in wells in an agarose gel.” Another caption reads, “An electrical current is passed through the gel.” A downward arrow labeled completion of migration leads to part “b” in which the electrophoretic chamber shows bands in the gel. The leftmost well shows size standards. Large fragments and small fragments are labeled on the bands. A caption reads, “All D N A fragments move toward the positive pole; small fragments migrate faster than large fragments. After electrophoresis, fragments of different sizes have migrated different distances.” A downward arrow leads to part “c” which shows a photo of the bands formed during gel electrophoresis. A caption reads, “A dye specifi¬c for nucleic acids is added to the gel.” Another caption reads, “D N A fragments appear as bands on the gel.”

The DNA fragments are still too small to see, so the problem of visualizing them must be addressed. Visualization can be accomplished in several ways. The simplest procedure is to stain the gel with a dye specific for nucleic acids, such as ethidium bromide, which wedges itself tightly (intercalates) between the bases of DNA and fluoresces when exposed to UV light, producing brilliant bands on the gel (Figure 19.4c). Alternatively, DNA fragments can be visualized by adding a label to the DNA before it is placed in the gel. For example, chemical labels can be detected by adding antibodies or other substances that carry a dye and will attach to the relevant DNA, which can then be visualized directly.

CONCEPTS

DNA fragments can be separated, and their sizes determined, with the use of gel electrophoresis. The fragments can be viewed by using a dye that is specific for nucleic acids or by labeling the fragments with a chemical tag.

✔ CONCEPT CHECK 2

DNA fragments that are 500 bp, 1000 bp, and 2000 bp in length are separated by gel electrophoresis. Which fragment will migrate farthest in the gel?

a. The 2000-bp fragment
b. The 1000-bp fragment
c. The 500-bp fragment
d. All will migrate equal distances.

Locating DNA Fragments with Probes

If a small piece of DNA, such as a plasmid, is cut by a restriction enzyme, the few fragments produced can be seen as distinct bands on an electrophoretic gel. In contrast, if genomic DNA from a cell is cut by a restriction enzyme, thousands of fragments of different sizes are produced. A restriction enzyme that recognizes a 4-base sequence would, theoretically, cut about once every 256 bp. The human genome, with 3.2 billion base pairs, would generate more than 12 million fragments when cut by this restriction enzyme. When separated by electrophoresis and stained, this large set of fragments would appear as a continuous smear on the gel because of the presence of so many fragments of differing sizes. Usually, researchers are interested in only a few of these fragments, perhaps

those carrying a specific gene. How can they locate the desired fragments in such a large pool of DNA?

One approach is to use a **probe**, which is often a DNA or RNA molecule with a base sequence complementary to a sequence in the gene of interest. The bases on the probe will pair only with the bases on a complementary sequence, so a suitably labeled probe can be used to locate a specific gene or an other DNA sequence. To use a probe, a researcher first cuts the DNA into fragments by using one or more restriction enzymes and then separates the fragments with gel electrophoresis. Next, the separated fragments must be denatured and transferred to a permanent solid medium (such as a nitrocellulose or nylon membrane). **Southern blotting** (named after Edwin M. Southern) is one technique used to transfer the denatured (single-stranded) fragments from a gel to a permanent solid medium.

After the single-stranded DNA fragments have been transferred, the membrane is placed in a hybridization solution containing a labeled probe. The probe binds to (hybridizes with) any DNA fragments on the membrane that bear complementary sequences. Often, a probe binds to only a part of a DNA fragment, so the DNA fragment may contain sequences not found in the probe. The membrane is then washed to remove any unbound probe; a biochemical method reveals the presence of the bound probe.

RNA can be transferred from a gel to a solid support by a related procedure called **Northern blotting** (not named after anyone but

capitalized to match Southern blotting). Hybridization with a probe can reveal the size of a particular mRNA molecule, its relative abundance, or the tissues in which it is transcribed. **Western blotting** is the transfer of protein from a gel to a membrane. Here, the probe is usually an antibody, used to determine the size of a particular protein and the pattern of the protein's expression.

CONCEPTS

Labeled probes, which are sequences of RNA or DNA that are complementary to the sequence of interest, can be used to locate individual genes or sequences among DNA fragments separated by electrophoresis.

✔ CONCEPT CHECK 3

How do Northern and Western blotting differ from Southern blotting?

19.3 Specific DNA Fragments Can Be Amplified

Many of the methods used to manipulate and analyze DNA sequences cannot be carried out on single molecules, requiring instead numerous copies of a specific DNA fragment. A major problem in working at the molecular level is that each gene is a tiny fraction of the total cellular DNA. Because each gene is so rare, it must be isolated and amplified before it can be studied. There are two basic approaches to amplifying a specific DNA fragment: replicating the DNA within cells (in vivo), or replicating the DNA enzymatically outside of cells (in vitro).

In the in vivo approach, a DNA fragment is inserted into a bacterial cell and the cell is allowed to replicate the DNA. Each time the cell divides, one or more copies of the DNA fragment are passed on to each daughter cell. Most bacterial cells divide rapidly, so within a short time (usually a few days), a large number of genetically identical cells are produced, each carrying one or more copies of the DNA fragment. The cells are then lysed to release their DNA, and the desired fragment is isolated from the rest of the bacterial DNA. This procedure is termed **gene cloning** because identical copies (clones) of the original piece of DNA are replicated within bacterial cells.

For many years, all amplification of DNA was done by gene cloning. A major disadvantage of gene cloning is the time required: the

process of inserting the DNA into bacteria, selecting and growing the bacterial cells that have incorporated it, and isolating the amplified DNA usually takes several days. Gene cloning is also relatively labor-intensive, requiring a number of steps that are difficult to automate. An advantage is that, because it uses the cell's high-fidelity replication machinery, gene cloning typically copies DNA with great accuracy.

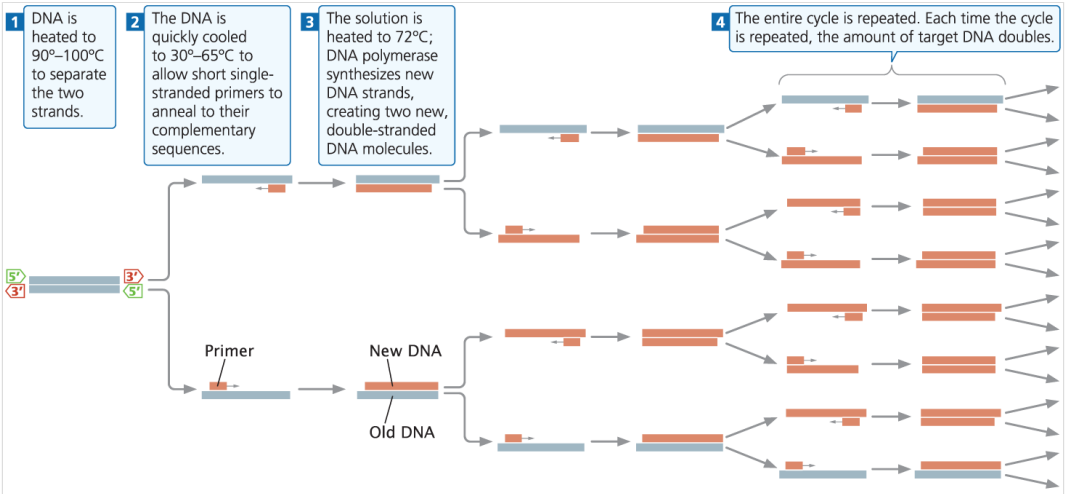
The second, in vitro approach is to amplify DNA enzymatically in a test tube outside cells. This amplification is done with the **polymerase chain reaction (PCR)**, a technique first developed in 1983 by Kary Mullis. The basis of PCR is DNA replication catalyzed by a DNA polymerase. Because a DNA molecule consists of two nucleotide strands, each of which can serve as a template, the amount of DNA doubles with each replication event. PCR allows DNA fragments to be amplified a billionfold within just a few hours, and it can be used with extremely small amounts of original DNA, even a single molecule. The polymerase chain reaction revolutionized molecular biology and is now one of the most widely used molecular techniques.

We first discuss PCR because it is the most widely used technique for amplifying DNA fragments today. We then return to gene cloning, which, in addition to its use in amplifying DNA, is often employed to manipulate gene sequences.

The Polymerase Chain Reaction

Replication by PCR requires a DNA template (the fragment of interest, or target DNA) from which a new DNA strand can be copied and a pair of single-stranded primers, each with a $3'$-OH group to which new nucleotides can be added. The primers used in PCR are short fragments of DNA, typically 17–25 nucleotides long, that are complementary to known sequences on the template. To carry out PCR, researchers begin with a solution that includes the target DNA, DNA polymerase, all four deoxyribonucleoside triphosphates (dNTPs—the substrates for DNA polymerase), primers, and magnesium ions and other salts that are necessary for the reaction to proceed.

A typical polymerase chain reaction includes three steps (**Figure 19.5**). In step 1, a starting solution of DNA is heated to 90°–100°C to break the hydrogen bonds between the strands and thus produce the necessary single-stranded templates. The reaction mixture is held at this temperature for only a minute or two. In step 2, the DNA solution is cooled quickly to 30°–65°C and held at this temperature for a minute or less. During this short interval, the DNA strands do not have a chance to reanneal, but the primers are able to attach to the template strands. In step 3, the solution is heated for a minute or less to 72°C, the temperature at which DNA polymerase can synthesize new DNA strands. At the end of the cycle, two double-stranded DNA molecules are produced for each original molecule of DNA.



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19.5 The polymerase chain reaction can be used to amplify even very small samples of DNA.

Description

The flowchart starts with a double stranded D N A with 5 prime and 3 prime labeled on both strands. The two strands separate and short primers are attached to their inner ends. A new D N A forms on both strands. The cycle repeats and produces eight D N A. Two D N A have one old D N A and one newly synthesized strand. The rest have both new strands. The captions in the illustration read, “first, D N A is heated to 90 to 100 degree Celsius to separate the two strands. Second, The D N A is quickly cooled to 30 to 65 degrees Celsius to allow short single-stranded primers to anneal to their complementary sequences. Third, the solution is heated to 72 degrees Celsius; D N A polymerase synthesizes new D N A strands, creating two new, double-stranded D N A molecules. Fourth, the entire cycle is repeated. Each time the cycle is repeated, the amount of target D N A doubles.”

The whole cycle is then repeated. With each cycle, the amount of target DNA doubles, so the amount of target DNA increases geometrically. One molecule of DNA increases to more than 1000 molecules in 10 PCR cycles, to more than 1 million molecules in 20 cycles, and to more than 1 billion molecules in 30 cycles. Each cycle

is completed within a few minutes, so a large amplification of DNA can be achieved within a few hours. To see how the polymerase chain reaction quickly increases the number of copies of a DNA fragment, view (A) **Animation 19.1**.

Two key innovations facilitated the use of PCR in the laboratory. The first was the discovery of a DNA polymerase that is stable at the high temperatures used in step 1. The DNA polymerase from *E. coli* that was originally used in PCR denatures at 90°C, so fresh enzyme had to be added to the reaction mixture in *each* cycle, which slowed the process considerably. This obstacle was overcome when DNA polymerase was isolated from the bacterium *Thermus aquaticus*, which lives in the boiling springs of Yellowstone National Park. This enzyme, dubbed ***Taq* polymerase**, is remarkably stable at high temperatures and is not denatured in the strand-separation step; it can be added to the reaction mixture at the beginning of the PCR process and will continue to function through many cycles.

The second key innovation was the development of automated thermal cyclers: machines that bring about the rapid temperature changes necessary for the different steps of PCR. Originally, tubes containing reaction mixtures were moved by hand among water baths set at the different temperatures required for the three steps of each cycle. In automated thermal cyclers, the reaction tubes are placed in a metal block whose temperature changes rapidly according to a computer program.

In addition to amplifying DNA, PCR can be used to amplify sequences corresponding to RNA. This amplification is accomplished by first converting RNA into complementary DNA (cDNA) with reverse transcriptase. The cDNA can then be amplified by the usual PCR. This technique is referred to as **reverse-transcription PCR**.

LIMITATIONS OF PCR

Today, the polymerase chain reaction is often used in place of gene cloning, but it has several limitations. First, the use of PCR requires prior knowledge of at least part of the sequence of the target DNA so that the primers can be constructed. Second, the capacity of PCR to amplify extremely small amounts of DNA makes contamination a significant problem. Minute amounts of DNA from the skin of laboratory workers or small particles in the air can enter a reaction tube and be amplified along with the target DNA. A careful laboratory technique and the use of controls are necessary to circumvent this problem.

A third limitation of PCR is accuracy. Unlike other DNA polymerases, *Taq* polymerase does not have the capacity to proofread (see Chapter 12), and under standard PCR conditions, it incorporates an incorrect nucleotide about once every 20,000 bp. Other heat-stable DNA polymerases with proofreading capacity have been isolated that give more accurate PCR results.

A fourth limitation of PCR is that the size of the fragments that can be amplified by standard *Taq* polymerase is usually less than 2000 bp. By using a combination of *Taq* polymerase and a DNA polymerase with proofreading capacity, and by modifying the reaction conditions, investigators have been successful in extending PCR amplification to larger fragments, but even these larger fragments are limited in length to 50,000 bp.

APPLICATIONS OF PCR

In spite of its limitations, PCR has become one of the most widely used tools within molecular biology. Because the primers used in PCR are specific for known DNA sequences, PCR can be used to detect the presence of a particular DNA sequence in a sample. For example, PCR is often used to detect the presence of viruses in blood samples by adding primers complementary to known viral DNA sequences to the reaction mixture. If viral DNA is present, the primers will attach to it, and a DNA fragment of a known length will be amplified. The presence of a DNA fragment of that length on a gel indicates the presence of viral DNA in the blood sample. Modern diagnostic tests for infection with HIV, the causative agent of AIDS (see Section 9.5), use this type of PCR amplification of HIV sequences.

The presence of DNA has been used to locate rare and secretive animals through the collection and analysis of **environmental DNA**

(eDNA). This has proven to be especially useful for aquatic and marine organisms. These animals are constantly shedding DNA into their aquatic or marine environment. Water from a river, a spring, or the ocean is collected and filtered for DNA. The eDNA is isolated, and primers specific to the genome of the organism of interest are used in a PCR reaction, which amplify that organism's DNA when it is present. The presence of a PCR product (usually a band on a gel) provides evidence that the organism is present in the sampled environment. The identification can be verified by sequencing the PCR product and determining that the sequence belongs to the organism of interest.

Another common application of PCR is to identify genetic variation in natural populations. PCR can be used to amplify specific segments of DNA, which are then analyzed with other molecular tools that identify differences in nucleotide sequences (see Chapter 26). In addition, primers specific to a particular DNA variant can be used to determine whether that variant is present in the genome of an individual organism.

PCR has also allowed the isolation of DNA from ancient sources, such as DNA from Neanderthals (discussed in Section 1.1). It is commonly used to amplify small samples of DNA from crime scenes and identify their sources through detection of microsatellite variation (see the discussion of DNA fingerprinting in Section 19.5).

PCR can be used to introduce new sequences into a fragment of DNA. Although the 3′ ends of the primers used in PCR must be complementary to the template, there can be flexibility in the sequences at the 5′ ends of the primers. Primers can be designed to contain new restriction sites or other desirable sequences at their 5′ ends. These sequences will be copied by PCR and thus added to the ends of the amplified DNA.

A modification of PCR, known as **real-time PCR (qPCR)**, is used to measure the starting amount of nucleic acid. In this procedure, PCR is used to amplify a specific DNA fragment, and a sensitive instrument is used to accurately determine the amount of DNA that is present in solution after each PCR cycle. A fluorescent probe that is specific to the DNA sequence of interest is frequently used in the reaction, so that only the DNA of interest is measured. The technique is called real-time PCR because the amount of DNA amplified is measured as the reaction proceeds.

Often, real-time PCR is combined with reverse-transcription PCR (RT-PCR) to measure the amount of mRNA in a sample, allowing biologists to precisely determine the level of gene expression in different cells and under different conditions; this method is referred to as qRT-PCR. For example, researchers interested in how gene expression changes in response to the administration of a drug often use real-time PCR to measure the amount of mRNA produced by specific genes in cells exposed to the drug. They can then

compare it with the amount of mRNA produced by the same genes in control cells with no drug exposure.

CONCEPTS

The polymerase chain reaction is an enzymatic, in vitro method for rapidly amplifying DNA. In this process, DNA is heated to separate the two strands, short primers attach to the target DNA, and DNA polymerase synthesizes new DNA strands from the primers. Each cycle of PCR doubles the amount of DNA. PCR has a number of important applications in molecular biology.

✔ CONCEPT CHECK 4

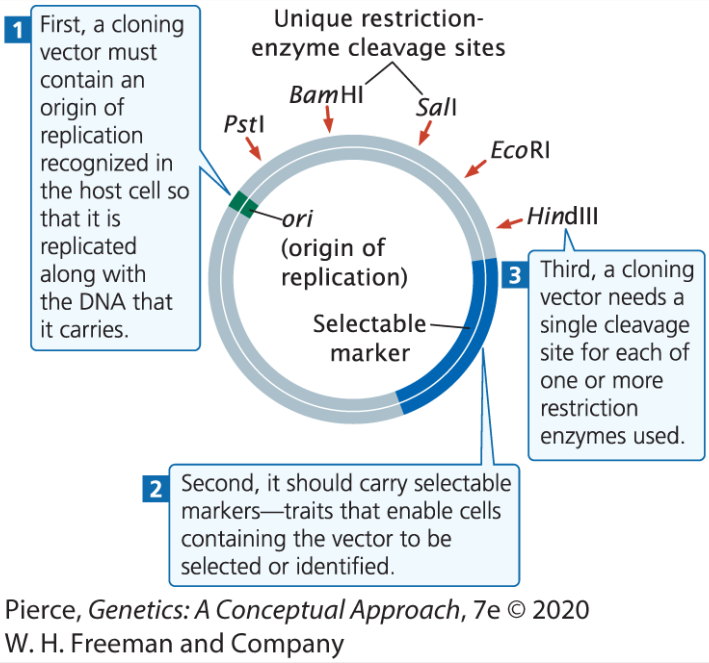
Why is the use of a heat-stable DNA polymerase important to the success of PCR?

Gene Cloning

Despite the widespread use of PCR, some DNA sequences are still amplified by gene cloning. In addition, gene cloning provides a powerful means of altering and manipulating DNA sequences. It is often used to alter cells so that they have desired properties or produce substances of commercial value. Here we outline some of the methods used to manipulate DNA sequences and introduce them into bacterial cells.

Many cloning techniques require a **cloning vector**: a stable, replicating DNA molecule to which a foreign DNA fragment can be

attached for introduction into a cell. An effective cloning vector has three important characteristics (Figure 19.6): (1) an origin of replication, which ensures that the vector is replicated within the cell; (2) selectable markers, which enable any cells containing the vector to be selected or identified; and (3) one or more unique restriction sites into which a DNA fragment can be inserted. The restriction sites used for cloning must be unique; if a vector is cut at multiple recognition sites, several pieces of DNA are generated, and getting those pieces back together in the correct order is possible but extremely difficult.



19.6 An ideal cloning vector has an origin of replication, one or more selectable markers, and recognition sites for one or more restriction enzymes.

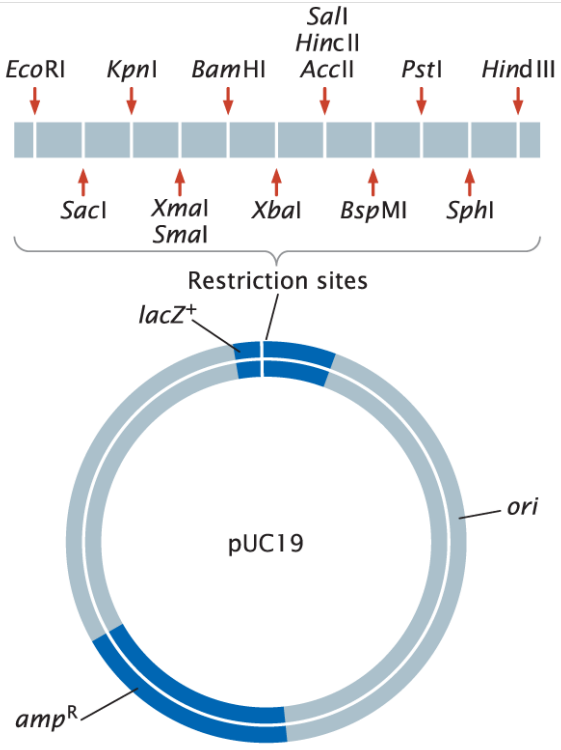
Description

The vector shows two concentric circles very close to one another. A short fragment on each circle shows Ori (origin of replication) on the top left of the vector. A large arc on the

circles on the bottom right is labeled selectable marker. In between ori and selectable marker, various unique restriction enzyme cleavage sites are present. Their sequence from left to right is as follow: P S T 1, Bam H 1, Sal 1, Eco R 1, and Hind 3. Captions in the vector map are as follows: “First, a cloning vector must contain an origin of replication recognized in the host cell so that it is replicated along with the D N A that it carries. Second, it should carry selectable markers—traits that enable cells containing the vector to be selected or identi¬fied. Third, a cloning vector needs a single cleavage site for each of one or more restriction enzymes used.”

PLASMID VECTORS

Plasmids—circular DNA molecules that exist naturally in bacteria (see Chapter 9)—are commonly used as vectors for cloning DNA fragments in bacteria. They contain origins of replication and are therefore able to replicate independently of the bacterial chromosome. The plasmids typically used in cloning have been artificially constructed from larger, naturally occurring bacterial plasmids and have multiple unique restriction sites and selectable markers, as well as an origin of replication (**Figure 19.7**).



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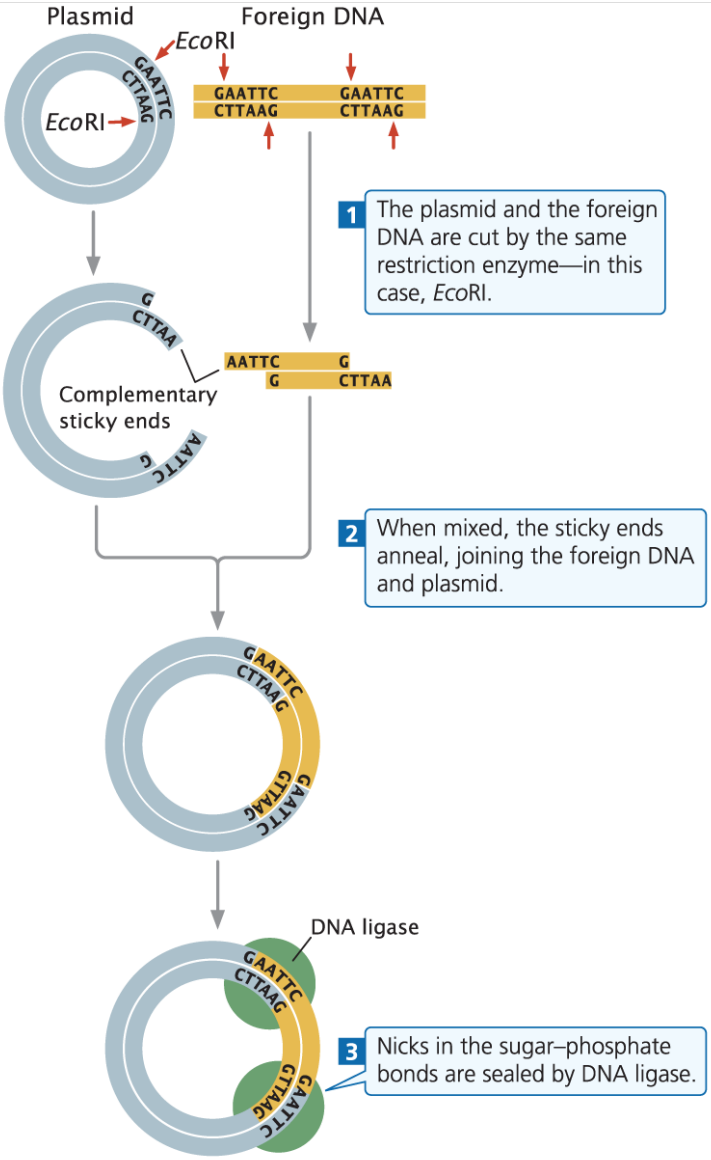
19.7 The pUC19 plasmid is a typical cloning vector. This artificially constructed plasmid contains a cluster of unique restriction sites, an origin of replication, and two selectable markers: an ampicillin-resistance gene and a *lacZ* gene.

Description

The vector shows two concentric circles very close to one another. A large segment of the circles on the right is labeled Ori. A small segment on bottom left is labeled amp superscript R. Another small segment on top of the circles is labeled restriction sites. The sequence of sites from left to right is as follows: Eco R 1, Sac 1, K P N 1, X m a 1, S m a 1, Bam H 1, X b a 1, S a l 1, H i n c 2, A c c 2, B s p M 1, P s t 1, S p h 1, and Hind 3. Lac Z superscript positive is also labeled on the restriction sites arc.

The easiest method for inserting a DNA sequence into a plasmid vector is to cut the foreign DNA (containing the DNA fragment of interest) and the plasmid with the same restriction enzyme (Figure

19.8). If the restriction enzyme makes staggered cuts in the DNA, complementary sticky ends are produced on the foreign DNA and the plasmid. When DNA and plasmids are then mixed together, some of the foreign DNA fragments will pair with the cut ends of the plasmids. DNA ligase is used to seal the nicks in the sugar–phosphate backbone, creating a recombinant plasmid that contains the foreign DNA fragment. You can learn more about plasmid cloning by viewing (A) **Animation 19.2**.



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19.8 A foreign DNA fragment can be inserted into a plasmid with the use of restriction enzymes.

Description

The illustration shows a circular plasmid with Eco R 1 restriction site as follows: first strand, G (an arrow points between) A A T T C; second strand C T T A A (an arrow points between) G. A linear double-stranded foreign D N A has the sequence, G (an arrow points between) A A T T C on the upper strand and C T T A A (an arrow points between) G on the lower strand on two sides. A caption reads, “The plasmid and the foreign D N A are cut by

the same restriction enzyme—in this case, Eco R 1." Complementary sticky ends are produced in the plasmid and the foreign D N A. The plasmid has the following sequence on one side: first strand, G; second strand, C T T A A and on the other side the sequence is as follows: first strand, A A T T C; second strand, G. The foreign D N A has the sequence A A T T C on one side and G on the other side of first strand and G on one side and C T T A A on the other side of the second strand. Another caption reads, "When mixed, the sticky ends anneal, joining the foreign D N A and plasmid." The complementary sticky ends are joined together and show a plasmid having the sequence G A A T T C on first strand and C T T A A G on second strand on two positions. D N A ligase is joined to the nicks on the plasmid. Another caption reads, "Nicks in the sugar–phosphate bonds are sealed by D N A ligase."

Sometimes restriction sites are not available at a place where the DNA needs to be cut. In that case, a restriction site can be created with the use of **linkers**, which are small synthetic DNA fragments that contain one or more restriction sites. Linkers can be attached to the ends of any piece of DNA and are then cut by a restriction enzyme, generating sticky ends that are complementary to sticky ends on the plasmid.

TRANSFORMATION

Once a DNA fragment of interest has been placed inside a plasmid, the plasmid must be introduced into bacterial cells. This task is usually accomplished by *transformation*, the mechanism by which bacterial cells take up DNA from the external environment (see Section 9.3). Some types of cells undergo transformation naturally; others must be treated chemically or physically before they will

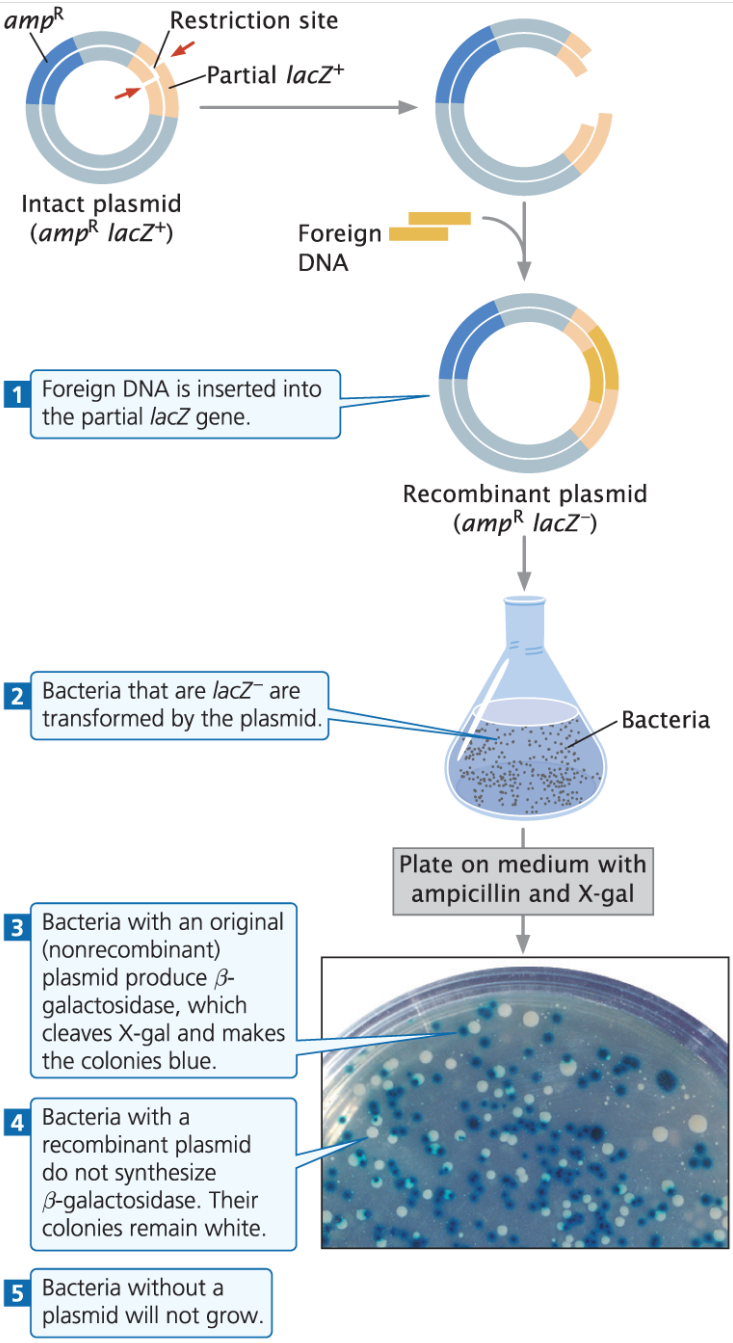
undergo transformation. Inside the cells, the plasmids replicate and multiply as the cells themselves multiply.

SCREENING CELLS FOR RECOMBINANT PLASMIDS

Cells bearing recombinant plasmids can be detected with the use of the selectable markers on the plasmid. Genes that confer resistance to an antibiotic are commonly used as selectable markers: any cell that contains such a gene will be able to live in the presence of the antibiotic, which normally kills bacterial cells.

One way to screen cells for the presence of a recombinant plasmid is to use a plasmid that contains a fragment of the *lacZ* gene—a small part of the front end of the gene (**Figure 19.9**). This partial *lacZ* gene contains a series of unique restriction sites into which a piece of DNA can be inserted. The bacteria that are to be transformed by the plasmid also have special features that make the presence of the recombinant plasmid evident: they are $lacZ^-$, missing the front end of the *lacZ* gene but containing its back end. Without the plasmid, these bacteria are unable to synthesize *β*-galactosidase. If no foreign DNA has been inserted into the partial *lacZ* gene of a plasmid taken up by a cell, the front end of the gene (provided by the plasmid) and the back end of the gene (provided by the bacterium) work together within the cell to produce *β*-galactosidase (see Section 16.2). If foreign DNA has been successfully inserted into the restriction site, it disrupts the front end of the *lacZ* gene, and *β*-galactosidase is *not*

produced. The plasmid also usually contains a selectable marker, which may be a gene that confers resistance to an antibiotic such as ampicillin.



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Photograph courtesy of Edvotek.

19.9 The *lacZ* gene can be used to screen for bacteria containing recombinant plasmids. An artificially constructed plasmid carries a fragment of the *lacZ* gene and an ampicillin-resistance gene.

Description
The illustration shows an intact circular plasmid (amp superscript R lac Z superscript positive) having a restriction site near the partial lac Z superscript positive gene. It is then cleaved on the restriction site and a complementary foreign D N A inserts in it, which is labeled recombinant plasmid (amp superscript R lac Z superscript positive). A caption reads, “Foreign D N A is inserted into the partial lac Z gene.” This is transferred into a conical flask containing bacteria. A caption reads, “Bacteria that are lac Z superscript negative are transformed by the plasmid.” The bacteria are then plated on medium with ampicillin and X-gal. A photo of bacterial culture with blue and white colonies in a petri dish is shown. The captions read as follows: Bacteria with an original (nonrecombinant) plasmid produce beta-galactosidase, which cleaves X-gal and makes the colonies blue. Bacteria with a recombinant plasmid do not synthesize beta-galactosidase. Their colonies remain white. Bacteria without a plasmid will not grow. Conclusion states that a white colony consists of bacteria carrying a recombinant plasmid.

Bacteria that are *lacZ*$^{-}$ are exposed to the plasmids and then plated on a medium that contains ampicillin. Only cells that have been successfully transformed and thus contain a plasmid with the ampicillin-resistance gene will survive and grow. Some of these cells contain an intact plasmid, whereas others possess a recombinant plasmid. The medium also contains the chemical X-gal, which produces a blue substance when cleaved. Bacterial cells with an intact original plasmid—without an inserted DNA fragment—have a functional *lacZ* gene (the front end of which is provided by the plasmid, and the back end by the bacterium). These bacteria can synthesize *β*-galactosidase, which cleaves X-gal and turns the bacteria blue. Bacterial cells with a recombinant plasmid, however, have had the front end of the *lacZ* gene disrupted by the inserted DNA; they do not synthesize *β*-galactosidase and remain white.

(Recall that the cells' own *lacZ* genes have been inactivated, so only bacteria with the plasmid turn blue.) Thus, the color of a bacterial colony allows quick determination of whether a recombinant or intact plasmid is present in the cells. After cells with the recombinant plasmid have been identified, they can be grown in large numbers to replicate the inserted fragment of DNA.

Plasmids make ideal cloning vectors, but they can hold only DNA fragments of less than about 15 kb in size. When large DNA fragments are inserted into a plasmid vector, the plasmid becomes unstable and tends to lose DNA spontaneously.

OTHER CLONING VECTORS

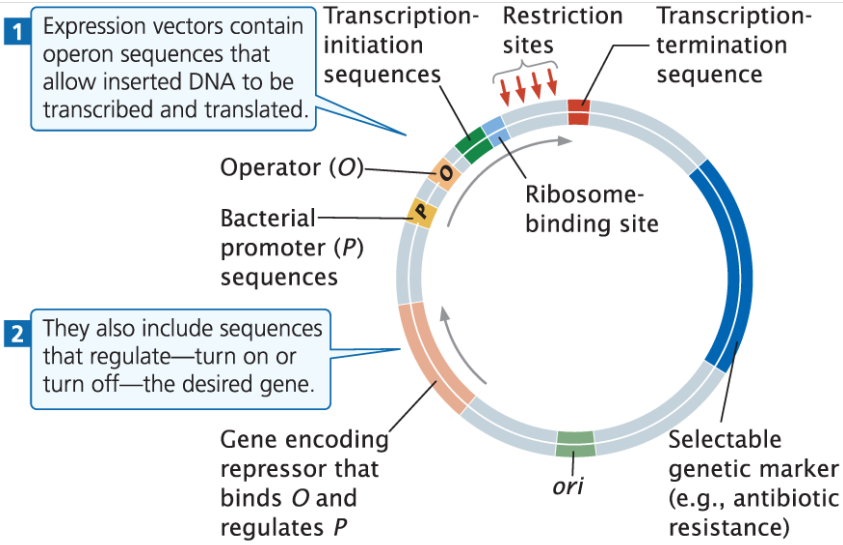
A number of other vectors have been developed for cloning larger pieces of DNA in bacteria. For example, bacteriophage λ, which infects *E. coli*, can be used to clone up to 23,000 bp of foreign DNA; it transfers DNA into bacterial cells with high efficiency. **Cosmids** are plasmids that are packaged in empty viral protein coats and transferred to bacteria by viral infection. They can carry almost twice as much foreign DNA as can a phage vector. **Bacterial artificial chromosomes (BACs)** are vectors originally constructed from the F plasmid (which controls mating and the transfer of genetic material in some bacteria; see Section 9.3) and can hold very large fragments of DNA (as long as 300,000 bp). **Table 19.3** compares the properties of plasmids, phage λ, cosmids, and BACs.

TABLE 19.3 Comparison of plasmids, phage λ vectors, cosmids, and bacterial artificial chromosomes

Cloning Vector	Size of DNA That Can Be Cloned	Method of Propagation	Introduction to Bacteria
Plasmid	As large as 15 kb	Plasmid replication	Transformation
Phage λ	As large as 23 kb	Phage reproduction	Phage infection
Cosmid	As large as 44 kb	Plasmid reproduction	Phage infection
Bacterial artificial chromosome	As large as 300 kb	Plasmid reproduction	Electroporation

Note: 1 kb = 1000 bp. Electroporation consists of electrical pulses that increase the permeability of a membrane.

Sometimes the goal in gene cloning is not just to replicate the gene but also to produce the protein it encodes. To ensure transcription and translation, a foreign gene is usually inserted into an **expression vector**, which—in addition to the usual origin of replication, restriction sites, and selectable markers—contains sequences required for transcription and translation in bacterial cells (**Figure 19.10**).



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19.10 To ensure transcription and translation, a foreign gene may be inserted into an expression vector. In this example, an *E. coli* expression vector has been used.

Description

The vector shows two concentric circles very close to one another. Ori (origin of replication) is labeled on the bottom center of the vector. A large arc on the circles on center right is labeled selectable marker, such as antibiotic resistance. On the bottom left, it shows a region with genes encoding repressor that binds O and regulates P. In between these genes and selectable marker, various sites are labeled. Their sequence from left to right is as follow: bacterial promoter sequences (P), operator (O), transcription initiation sequences, multiple restriction sites, and the transcription-termination sequence. Captions in the illustration read as follows: “Expression vectors contain operon sequences that allow inserted D N A to be transcribed and translated. They also include sequences that regulate —turn on or turn off—the desired gene.”

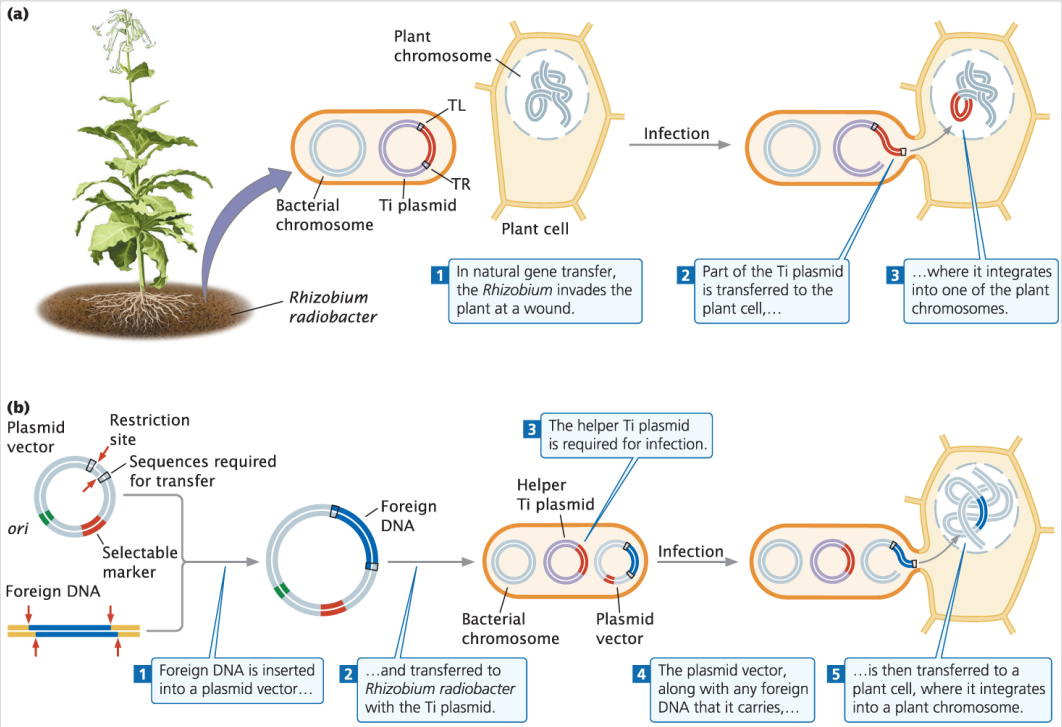
Although manipulating genes in bacteria is simple and efficient, the goal may be to transfer a gene into eukaryotic cells. For example, it might be desirable to transfer a gene conferring herbicide resistance

into a crop plant, or to transfer a gene for clotting factor into a person with hemophilia. Many eukaryotic proteins are modified after translation (e.g., carbohydrate groups may be added). Such modifications are essential for proper function, but bacteria do not have the capacity to carry them out; thus, a functional protein can be produced only in a eukaryotic cell.

A number of cloning vectors have been developed that allow the insertion of genes into eukaryotic cells. Special plasmids have been developed for cloning in yeast, and retroviral vectors have been developed for cloning in mammals. A **yeast artificial chromosome (YAC)** is a DNA molecule that has a yeast origin of replication, a pair of telomeres, and a centromere. These features ensure that YACs are stable and that they replicate and segregate in the same way as yeast chromosomes. YACs are particularly useful because they can carry DNA fragments as large as 600 kb, and some special YACs can carry inserts of more than 1000 kb. YACs have been modified so that they can be used in eukaryotic organisms other than yeast.

The soil bacterium *Rhizobium radiobacter* (formerly known as *Agrobacterium tumefaciens*), which invades plants through wounds and induces crown galls (tumors), has been used to transfer genes to plants. This bacterium contains a large plasmid called the **Ti plasmid**, part of which is transferred to a plant cell when *R. radiobacter* infects a plant. In the plant, part of the Ti plasmid DNA integrates into one of the plant chromosomes, where it is

transcribed and translated to produce several enzymes that help support the bacterium (Figure 19.11a).



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19.11 The Ti plasmid can be used to transfer genes into plants. Flanking sequences TL and TR are required for the transfer of the DNA segment from bacteria to the plant cell.

Description

Part “a” shows a plant and soil around the roots is labeled Rhizobium radiobacter. From the roots, an arrow leads to the bacterial cell in which a circular double-stranded bacterial chromosome and a circular T i plasmid are labeled. An arc on the plasmid is shown in red. One end of the plasmid is labeled T L and the other T R. A plant cell with plant chromosome is then shown. Infection occurs and the red segment of the T i plasmid moves into the plant cell and incorporates into its chromosome. Captions read as follows: In natural gene transfer, the Rhizobium invades the plant at a wound. Part of the T i plasmid is transferred to the plant cell, where it integrates into one of the plant chromosomes.

Part “b” shows a circular plasmid vector with ori, restriction site, a sequence for required transfer, and selectable marker. A foreign D N A is also shown with a blue segment at the center and arrows at its terminal points. This leads to a recombinant vector with a foreign D N A having the blue segment in it. A caption reads, “Foreign D N A is inserted into a plasmid vector and transferred to Rhizobium radiobacter with the T i plasmid.” This plasmid vector is then inserted into a bacterial cell having bacterial chromosome and a helper T i plasmid. A caption reads, “The helper T i plasmid is required for infection.” Infection occurs then and the plasmid vector transfers foreign D N A into a plant cell which incorporates into the plant chromosome. A caption reads, “The plasmid vector, along with any foreign D N A that it carries, is then transferred to a plant cell, where it integrates into a plant chromosome.”

Geneticists have engineered a vector that contains the flanking sequences required to transfer DNA (called TL and TR; see Figure 19.11a), a selectable marker, and restriction sites into which foreign DNA can be inserted (**Figure 19.11b**). When placed in *R. radiobacter* with a helper Ti plasmid (which carries sequences allowing the bacterium to infect the plant cell, which the artificial vector lacks), this vector will transfer the foreign DNA that it carries into a plant cell, where that DNA will integrate into a plant chromosome. This vector has been used to transfer genes that confer economically significant attributes, such as resistances to herbicides, plant viruses, and insect pests.

THE GENETIC ENGINEERING OF PLANTS WITH PESTICIDES

An early and economically important use of the Ti cloning plasmid was the genetic engineering of plants that produce their own

insecticides. The bacterium *Bacillus thuringiensis* naturally produces a protein, known as the Bt toxin, that is lethal to many insects. The Bt toxin is particularly attractive as an insecticide because it is specific to particular insects, breaks down quickly in the environment, and is not toxic to humans and other animals. In 1987, Mark Vaeck and his colleagues at Plant Genetic Systems in Belgium genetically engineered tobacco plants to express the Bt toxin. Subsequently, other researchers introduced the gene for Bt toxin into cotton, tomatoes, corn, and other plants (Figure 19.12). Today, transgenic plants expressing the Bt toxin are used broadly throughout the world.

John L. Obermeyer, Purdue Extension Entomology.

19.12 Transgenic crops are broadly cultivated throughout the world. Shown here is Bt corn and unmodified corn growing in alternate strips, a strategy used to try to prevent the development of resistance to the genetically engineered Bt toxin by pests.

Because *R. radiobacter* cannot infect many plants of agricultural importance, other methods have been used to transfer genes into

these plants, including physically shooting small metal particles coated with DNA into cells with a "gene gun." Genes in plants are now routinely altered using TALENs and CRISPR-Cas systems (see Section 19.2). ❯ **TRY PROBLEM 39**

THINK-PAIR-SHARE Question 5

CONCEPTS

DNA fragments can be inserted into cloning vectors, stable pieces of DNA that will replicate within a cell. A cloning vector must have an origin of replication, one or more unique restriction sites, and selectable markers. An expression vector contains additional sequences that allow a cloned gene to be transcribed and translated. Special cloning vectors have been developed for introducing genes into eukaryotic cells.

✔ CONCEPT CHECK 5

How is a gene inserted into a plasmid cloning vector?

19.4 Molecular Techniques Can Be Used to Find Genes of Interest

To analyze a gene or to transfer it to another organism, the gene must be located and isolated. Today, most genes are located by sequencing a genome (see Chapter 20) and determining the locations of genes from the sequence. Before the development of low-cost sequencing methods, genes were often located by first creating libraries of DNA sequences and then screening those libraries for genes of interest. This approach—to clone first and search later—is called *shotgun cloning* because it is like hunting with a shotgun: the pellets spray widely in the general direction of the target, with a good chance that one or more of the pellets will hit it. In shotgun cloning, a researcher first clones a large number of DNA fragments, knowing that one or more contains the DNA of interest, and then searches for the fragment of interest among the clones.

DNA Libraries

A collection of clones containing all the DNA fragments from one source is called a **DNA library**. For example, we might isolate genomic DNA from human cells, break it into fragments, insert the fragments into vectors, and clone them in bacterial cells. The set of bacterial colonies or phages containing these fragments is a human

genomic library, containing all the DNA sequences found in the human genome.

A genomic library must contain a large number of clones to ensure that all DNA sequences in the genome are represented in the library. A library of the human genome formed by using cosmids, each carrying a random DNA fragment from 35,000 to 44,000 bp long, would require about 350,000 cosmid clones to provide a 99% chance that every sequence is included in the library.

An alternative to creating a genomic library is to create a library consisting only of those DNA sequences that are transcribed into mRNA (called a **cDNA library** because all the DNA in this library is *complementary* to mRNA). Much of eukaryotic DNA consists of sequences that are not transcribed into mRNA, and these sequences are not represented in a cDNA library. A cDNA library has two additional advantages. First, it is enriched with fragments from actively transcribed genes. Second, introns do not interrupt the cloned sequences; introns pose a problem when the goal is to produce a eukaryotic protein in bacteria, because most bacteria have no means of removing the introns. The disadvantage of a cDNA library is that it contains only sequences that are present in mature mRNA; therefore, sequences that are not transcribed, such as those in promoters and enhancers, are not present in a cDNA library. Furthermore, a cDNA library contains only those genes expressed in the tissue from which the RNA was isolated, and the frequency of a particular DNA sequence in a cDNA library depends on the

abundance of the corresponding mRNA in that tissue. In contrast, almost all genes are present at the same frequency in a genomic library.

To create a cDNA library, messenger RNA is first extracted from cells and separated from other types of cellular RNA (such as tRNA, rRNA, and snRNA). The mRNA molecules are then copied into cDNA using reverse transcriptase, an enzyme isolated from retroviruses (see Section 9.4) that synthesizes single-stranded complementary DNA using RNA as a template. The resulting RNA–DNA hybrid molecule is finally converted into a double-stranded cDNA molecule by DNA polymerase. The cDNA is then inserted into vectors and cloned in bacterial cells. › **TRY PROBLEM 43**

CONCEPTS

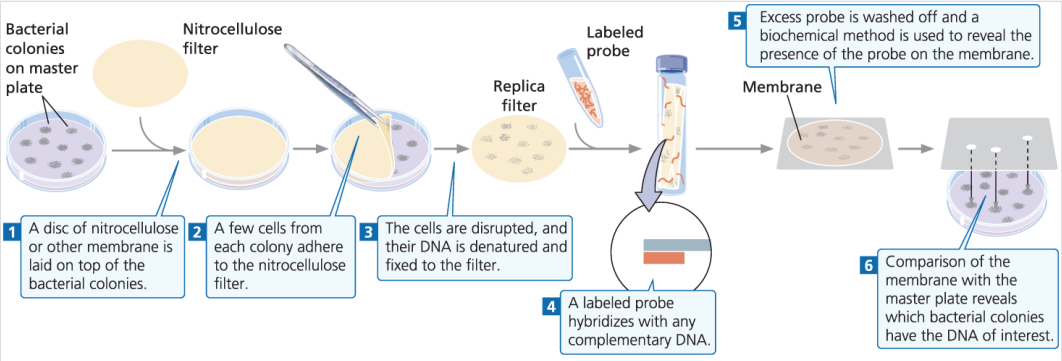
One method of finding a gene is to create and screen a DNA library. A genomic library is created by cutting genomic DNA into overlapping fragments and cloning each fragment in a separate bacterial cell. A cDNA library is created from mRNA that is converted into cDNA and cloned in bacteria.

SCREENING DNA LIBRARIES

Once a DNA library is created, it can be screened to find a gene or sequence of interest. The screening procedure used depends on what is known about the gene.

The first step in screening is to plate the clones of the library. If a plasmid or cosmid vector was used to construct the library, the bacterial cells are diluted and plated so that each bacterium grows into a distinct colony. If a phage vector was used, the phages are allowed to infect a lawn of bacteria on a petri plate. Each resulting plaque or bacterial colony contains a single cloned DNA fragment that must be screened for the gene of interest.

A common way to screen DNA libraries is with probes (Figure 19.13). But how is a probe obtained when the gene of interest has not yet been isolated? One option is to use a similar gene from another organism as the probe. For example, if we wanted to screen a human genomic library for the growth-hormone gene, and a growth-hormone gene had already been isolated from rats, we could use a purified rat-gene sequence as a probe to find the human gene for growth hormone. Successful hybridization does not require perfect complementarity between the probe and the target sequence, so a related sequence can often be used as a probe.



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19.13 Genomic and cDNA libraries can be screened with a probe to find the gene of interest.

Description
The illustration shows bacterial colonies on master plate. Nitrocellulose filter is then applied on top of the master plate. A caption reads, “A disc of nitrocellulose or other membrane is laid on top of the bacterial colonies. A disc of nitrocellulose or other membrane is laid on top of the bacterial colonies.” Then, the filter is removed and now the filter is labeled replica filter and it also shows bacterial colonies. Two captions read as follows: “A few cells from each colony adhere to the nitrocellulose filter. The cells are disrupted, and their D N A is denatured and fixed to the filter.” A labeled probe is then inserted and put it in a test tube. A magnified image shows two horizontal bands. One of them is shorter and of a different color. A corresponding caption reads, “A labeled probe hybridizes with any complementary D N A.” An arrow from the test tube leads to a membrane with colonies. A caption reads, “Excess probe is washed off and a biochemical method is used to reveal the presence of the probe on the membrane.” Then, the colonies are compared with the master plate. A caption reads, “Comparison of the membrane with the master plate reveals which bacterial colonies have the D N A of interest.”

Alternatively, synthetic probes can be created if the protein produced by the gene of interest has been isolated and its amino acid sequence has been determined. With the use of the genetic code, possible nucleotide sequences of a small region of the gene can be deduced from the amino acid sequence of the protein. Although only one nucleotide sequence in the gene encodes a particular protein, the existence of synonymous codons means that the same protein could be produced by several different sequences, and it is impossible to know which one is correct. To overcome this problem, a mixture of all the possible nucleotide sequences is used as a probe. To minimize the number of sequences required in the mixture, a region of the protein with relatively little degeneracy in its codons is selected. When part of the DNA sequence of the gene has

been determined, a set of DNA probes can be synthesized chemically by using an automated machine known as an oligonucleotide synthesizer.

Yet another method of screening a DNA library is to look for the protein product of a gene. This method requires that the DNA library be cloned in an expression vector. The clones can be tested for the presence of the protein by using an antibody that recognizes the protein or by using a chemical test for the protein. This method depends on the existence of an antibody or test for the protein produced by the gene of interest. DNA libraries can also be screened using PCR or by sequencing, as mentioned earlier in this chapter. To see how libraries are made and screened, view (A) **Animation 19.3**.

CONCEPTS

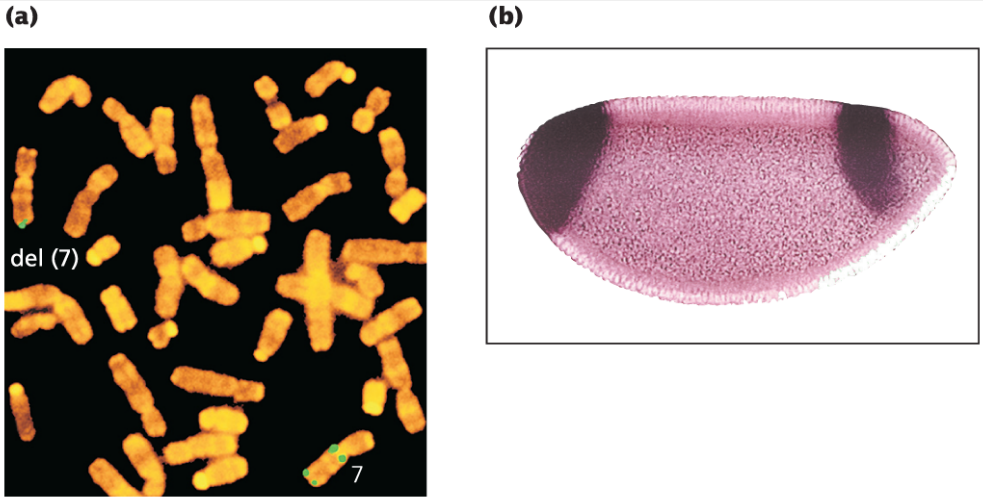
A DNA library can be screened for a specific gene with the use of complementary probes that hybridize to the gene. Alternatively, the library can be cloned in an expression vector, and the gene can be located by examining the clones for the protein product of the gene.

✔ CONCEPT CHECK 6

Briefly explain how synthetic probes are created to screen a DNA library when the protein encoded by the gene is known.

In Situ Hybridization

DNA probes can be used to determine the chromosomal location of a gene in a technique called **in situ hybridization**. The name of this technique is derived from the fact that DNA (or RNA) is visualized while it is in the cell (in situ). It requires that the cells be fixed and the chromosomes be spread on a microscope slide and denatured. A labeled probe is then applied to the slide, just as it can be applied to a gel. In fluorescence in situ hybridization (FISH), the probes carry attached fluorescent dyes that can be seen directly with the microscope (**Figure 19.14a**). Several probes attached to different colored dyes can be used simultaneously to investigate different sequences or chromosomes. FISH has been used to identify the chromosomal location of human genes.



Part a: Addenbrookes Hospital/Science Source. Part b: Courtesy of L.Tsuda.

19.14 With in situ hybridization, DNA probes are used to determine the chromosomal or cellular location of a gene or its product. (a) A probe attached to a green fluorescent dye is specific to a region of chromosome 7, revealing a deletion on one copy of chromosome 7. (b) In situ hybridization is used to detect the presence of mRNA from the *tailless* gene in a *Drosophila* embryo.

In situ hybridization can also be used to determine the distribution of specific mRNA molecules in tissues, serving as a source of insight into how gene expression differs among cell types (**Figure 19.14b**). A labeled DNA probe complementary to a specific mRNA molecule is added to tissue, and the location of the bound probe is determined by visualizing the fluorescent label. Determining where a gene is expressed often helps define its function. For example, finding that a gene is highly expressed only in brain tissue might suggest that the gene has a role in neural function.

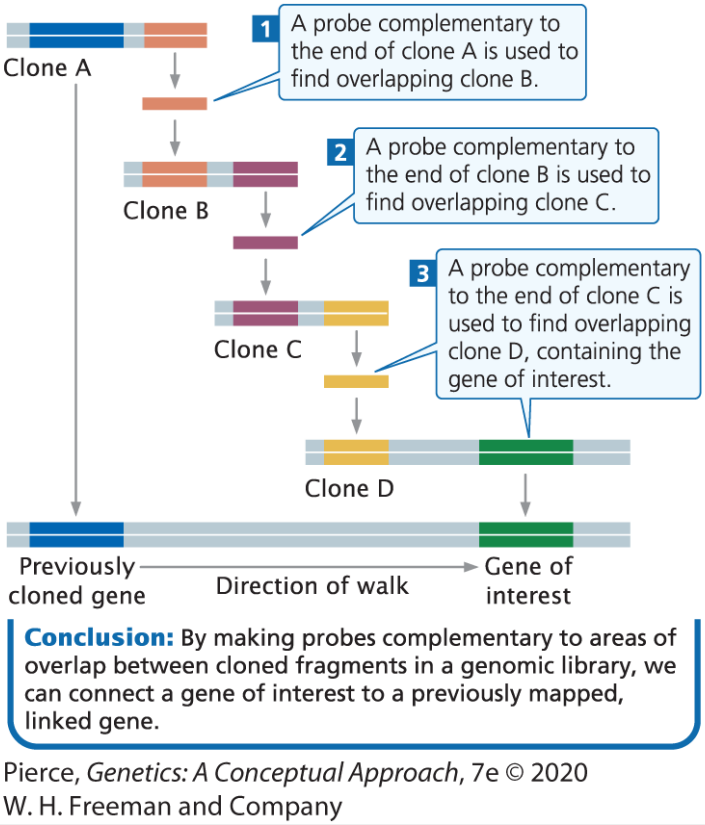
Positional Cloning

For many genes with important functions, no associated protein product has yet been identified. The underlying biochemical nature of many human genetic diseases, for example, is still unknown. How can these genes be isolated? One approach is to first determine the general location of the gene on a chromosome by using recombination frequencies derived from crosses or pedigrees (see Chapter 7). After the chromosomal region where the gene is found has been pinpointed, genes in that region can be cloned and identified. Then other techniques can be used to determine which of the "candidate" genes might be the one that causes the disease. This approach—isolation of genes on the basis of their position on a gene map—is called **positional cloning**.

In the first step of positional cloning, geneticists use mapping studies (see Chapter 7) to establish linkage between molecular markers and a phenotype of interest, such as a human disease or a desirable physical trait in a plant or an animal. Demonstration of linkage between the phenotype and one or more molecular markers tells us which chromosome carries the locus that codes for the phenotype and its general location on that chromosome.

The next step is to narrow down the location of the gene by using additional molecular markers clustered in the chromosomal region where the gene resides. Clones that cover that region can then be isolated from a genomic library. With the use of a technique called **chromosome walking** (**Figure 19.15**), it is possible to progress from clones of molecular markers to linked clones, one of which might contain the gene of interest. The basis of chromosome walking is the fact that a genomic library consists of a set of *overlapping* DNA fragments. We start with a cloned gene marker that is close to the new gene of interest so that the "walk" will be as short as possible. One end of the clone of a neighboring marker (clone A in Figure 19.15) is used to make a complementary probe. This probe is used to screen the genomic library to find a second clone (clone B) that overlaps with the first and extends in the direction of the gene of interest. This second clone is isolated and purified, and a probe is prepared from its end. The second probe is used to screen the library for a third clone (clone C) that overlaps with the second. In this way, one can systematically "walk" toward the gene of interest, one clone at a time. A related technique, called chromosome

jumping, allows one to move from more distantly linked markers to clones that contain a sequence of interest.



19.15 In chromosome walking, neighboring genes are used to locate a gene of interest.

Description

The illustration shows a double-stranded clone A which leads to a probe, which further leads to clone B. It further leads to another probe which leads to clone C and then to clone D. Both the strands of all the cloned genes have four segments; two of same color and two of different colors. The right end color of the previous clone is the same color of the next probe and the left end color of the upcoming clone. Finally, it leads to a large double stranded D N A. Two extreme regions are colored again. The left end color is the same as the left end color of clone A and is labeled “previously cloned gene” and the right end color is the same as the right end color of clone D and is labeled “gene of interest”. A forward arrow on the D N A reads direction of walk. Captions in the illustration are as follows: “A

probe complementary to the end of clone A is used to find overlapping clone B. A probe complementary to the end of clone B is used to find overlapping clone C. A probe complementary to the end of clone C is used to find overlapping clone D, containing the gene of interest." Conclusion states that by making probes complementary to areas of overlap between cloned fragments in a genomic library, we can connect a gene of interest to a previously mapped, linked gene.

After clones that cover the delineated chromosome region have been obtained by chromosome walking or jumping, all genes located within the region are identified. Genes can be distinguished from other sequences by the presence of characteristic features, such as consensus sequences in the promoter and a start codon and a stop codon within the same reading frame. After candidate genes have been identified, they can be evaluated to determine which is most likely to be the gene of interest. The expression pattern of the gene—where and when it is transcribed—can often provide clues about its function; for example, genes for neurological disease would probably be expressed in the brain. Geneticists often look in the coding regions of candidate genes for mutations among individuals with the phenotype of interest. The first gene responsible for a human genetic disease that was isolated entirely by positional cloning was the gene for cystic fibrosis.

CONCEPTS

Positional cloning allows researchers to isolate a gene without having knowledge of the biochemical basis of its phenotype. Linkage studies are used to map the locus producing a phenotype of interest to a particular chromosome region. Chromosome walking and jumping can be used to progress from molecular markers to clones containing sequences that cover the

chromosome region. Candidate genes within the region are then evaluated to determine whether they encode the phenotype of interest.

✔ CONCEPT CHECK 7

How are candidate genes that are identified by positional cloning evaluated to determine whether they encode the phenotype of interest?

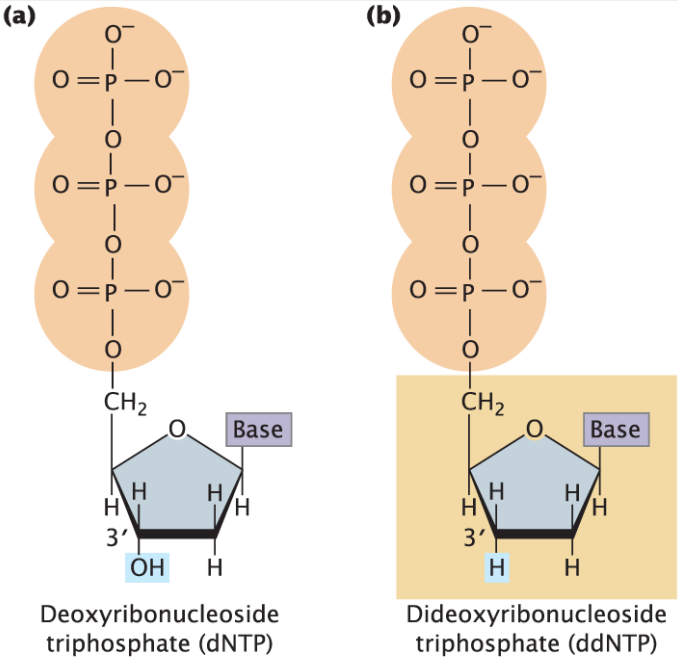
19.5 DNA Sequences Can Be Determined and Analyzed

A powerful molecular method for analyzing DNA is **DNA sequencing**, which determines the sequence of bases in a DNA molecule. Sequencing allows the genetic information in DNA to be read, providing an enormous amount of information about gene structure and function. In the mid-1970s, Frederick Sanger and his colleagues created the dideoxy-sequencing method, which is based on the elongation of DNA by DNA polymerase; at about the same time, Allan Maxam and Walter Gilbert developed a second method based on chemical degradation of DNA. The Sanger method quickly became the standard procedure for sequencing any purified fragment of DNA.

Dideoxy Sequencing

The dideoxy (or Sanger) method of DNA sequencing is based on replication. The fragment to be sequenced is used as a template to make a series of new DNA molecules. In the process, replication is sometimes, but not always, terminated when a specific base is encountered, producing DNA strands of different lengths, each of which ends in a known base.

The method relies on the use of a special substrate for DNA synthesis. Normally, DNA is synthesized from deoxyribonucleoside triphosphates (dNTPs), which have an OH group on the 3′-carbon atom (Figure 19.16a). In the Sanger method, special nucleotides, called dideoxyribonucleoside triphosphates (ddNTPs; Figure 19.16b), are also used as substrates. The ddNTPs are identical to dNTPs except that they lack a 3′-OH group. In the course of DNA synthesis, ddNTPs are incorporated into a growing DNA strand. However, after a ddNTP has been incorporated into the DNA strand, no more nucleotides can be added because there is no 3′-OH group to form a phosphodiester bond with an incoming nucleotide. Thus, ddNTPs terminate DNA synthesis.



19.16 The dideoxy-sequencing reaction requires a special substrate for DNA synthesis. (a) Structure of deoxyribonucleoside triphosphate, the normal

substrate for DNA synthesis. (b) Structure of dideoxyribonucleoside triphosphate, which lacks an OH group on the 3′-carbon atom.

Description
Diagram “a” shows the structure of deoxyribonucleoside triphosphate (d N T P). It shows a base attached to a pentose deoxyribose sugar. The chemical structure of pentose sugar is as follows: O attached to base, C H, C H 2, C H O H, C H 2 C H. From the carbon of C H 2 C H, three phosphate groups are attached in series, single bonded to each other. The P of these phosphate groups are labeled, alpha, beta, and gamma respectively as they move away from the C of C H 2 C H. The chemical structure of phosphate group is as follows: P is at the center. Four oxygen atoms are attached to it; one through double bond and three through single bonds. One of the single bonded O atoms has a negative charge. Diagram “b” shows the structure of dideoxyribonucleoside triphosphate (d d N T P). It is similar to d N T P, except it has H rather than O H in the pentose sugar.

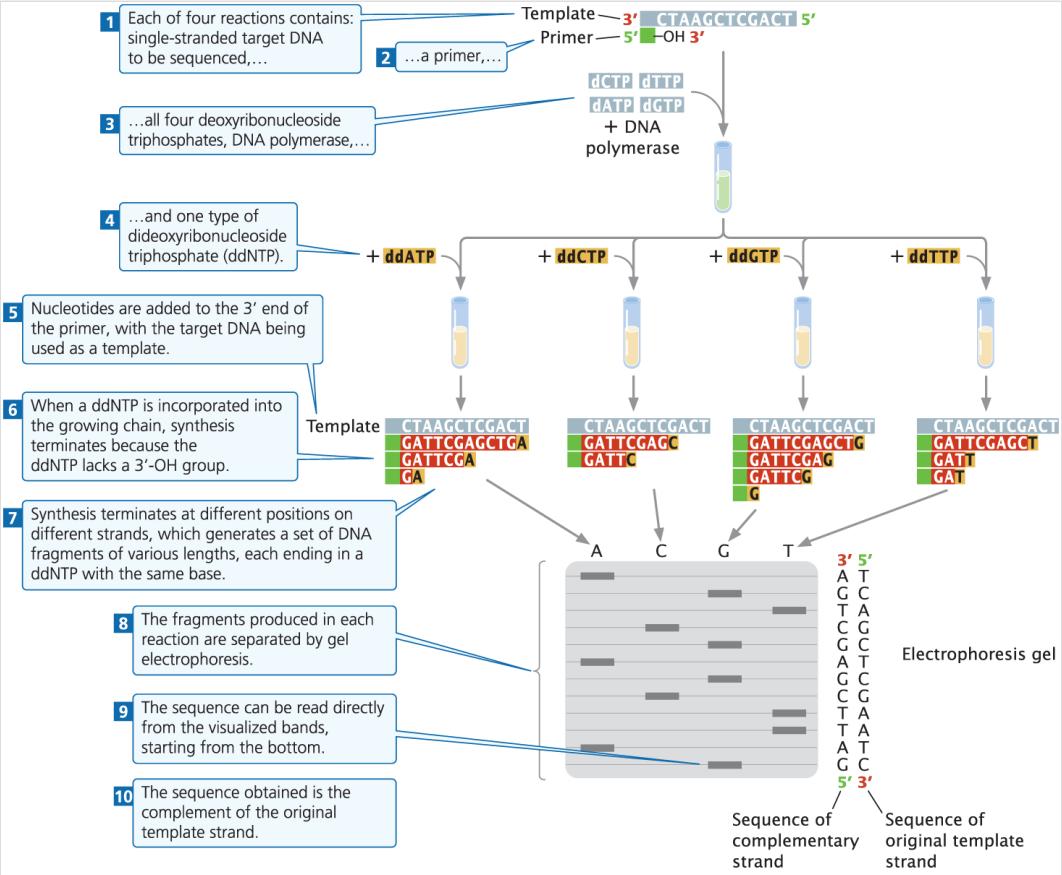
Although the sequencing of a single DNA molecule is technically possible, most sequencing procedures in use today require a considerable amount of DNA; thus, any DNA fragment to be sequenced must first be amplified by PCR or by cloning in bacteria. Copies of the target DNA are then isolated and split into four samples (Figure 19.17). Each sample is placed in a different tube, to which the following ingredients are added:

1. Many copies of a primer that is complementary to one end of the target DNA strand
2. All four types of deoxyribonucleoside triphosphates, the normal precursors of DNA synthesis
3. A small amount of one of the four types of dideoxyribonucleoside triphosphates (ddATP, ddCTP, ddGTP, or ddTTP), which will terminate DNA synthesis as soon as it is

incorporated into any growing chain (each of the four tubes receives a different ddNTP)

4. DNA polymerase

Either the primer or one of the dNTPs is radioactively or chemically labeled so that newly produced DNA can be detected.



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19.17 The dideoxy method of DNA sequencing is based on the termination of DNA synthesis.

Description

The illustration shows a template strand with sequence: 3 prime-C T A A G C T C G A C T-5 prime attached to a primer with O H at the 3-prime end. d N T P's plus D N A polymerase are added with the template and primer in a test tube. From this test tube, four separate

test tubes branch. One has d d A T P, one has d d C T P, one has d d G T P, and one has d d T T P. These lead to four different sequences with the template strand as follows: Test tube with d d A T P shows four strands as follows: 1, template; 2, G A T T C G A G C T G A; 3, G A T T C G A; 4, G A. Test tube with d d C T P shows three strands as follows: 1, template; 2, G A T T C G A G C; 3, G A T T C. Test tube with d d G T P shows five strands as follows: 1, template; 2, G A T T C G A G C T G; 3, G A T T C G A G; 4, G A T T C G; 5, G. Test tube with d d T T P shows four strands as follows: 1, template; 2, G A T T C G A G C T; 3, G A T T; 4, G A T. All these strands lead to a sequence of bands formed on gel electrophoresis. The heading reads, “A, C, G, and T”. The right vertical axis of the band sequence shows the sequence of complementary strand followed by the sequence of original template strand. The bands are shown from top to bottom as follows: A G T C G A G C T T A G. The captions in the illustration read as follows: “Each of four reactions contains: single-stranded target D N A to be sequenced, a primer, all four deoxyribonucleoside triphosphates, D N A polymerase, and one type of dideoxyribonucleoside triphosphate (d d N T P). Nucleotides are added to the 3 prime end of the primer, with the target D N A being used as a template. When a d d N T P is incorporated into the growing chain, synthesis terminates because the d d N T P lacks a 3 prime-O H group. Synthesis terminates at different positions on different strands, which generates a set of D N A fragments of various lengths, each ending in a d d N T P with the same base. The fragments produced in each reaction are separated by gel electrophoresis. The sequence can be read directly from the visualized bands, starting from the bottom. The sequence obtained is the complement of the original template strand.”

Within each of the four tubes, the DNA polymerase synthesizes DNA. Let’s consider the reaction in one of the four tubes: the one that received ddATP. Within this tube, each of the single strands of target DNA serves as a template for DNA synthesis. The primer pairs with its complementary sequence at one end of each template strand, providing a $3'$-OH group for the initiation of DNA synthesis. DNA polymerase elongates a new strand of DNA from this primer. Wherever DNA polymerase encounters a T on the template strand, it

uses, at random, either a dATP or a ddATP to introduce an A into the newly synthesized strand. Because there is more dATP than ddATP in the reaction mixture, dATP is incorporated most often, allowing DNA synthesis to continue. Occasionally, however, ddATP is incorporated into the strand, and synthesis terminates. The incorporation of ddA into a new strand occurs randomly at different positions in different copies, producing a set of DNA fragments of different lengths (12, 7, and 2 nucleotides long in the example illustrated in Figure 19.17), each ending in a nucleotide that contains adenine.

Equivalent reactions take place in the other three tubes except that synthesis is terminated at nucleotides with a different base in each tube. After the completion of the polymerization reactions, all the DNA in the tubes is denatured, and the single-stranded products of each reaction are separated by gel electrophoresis.

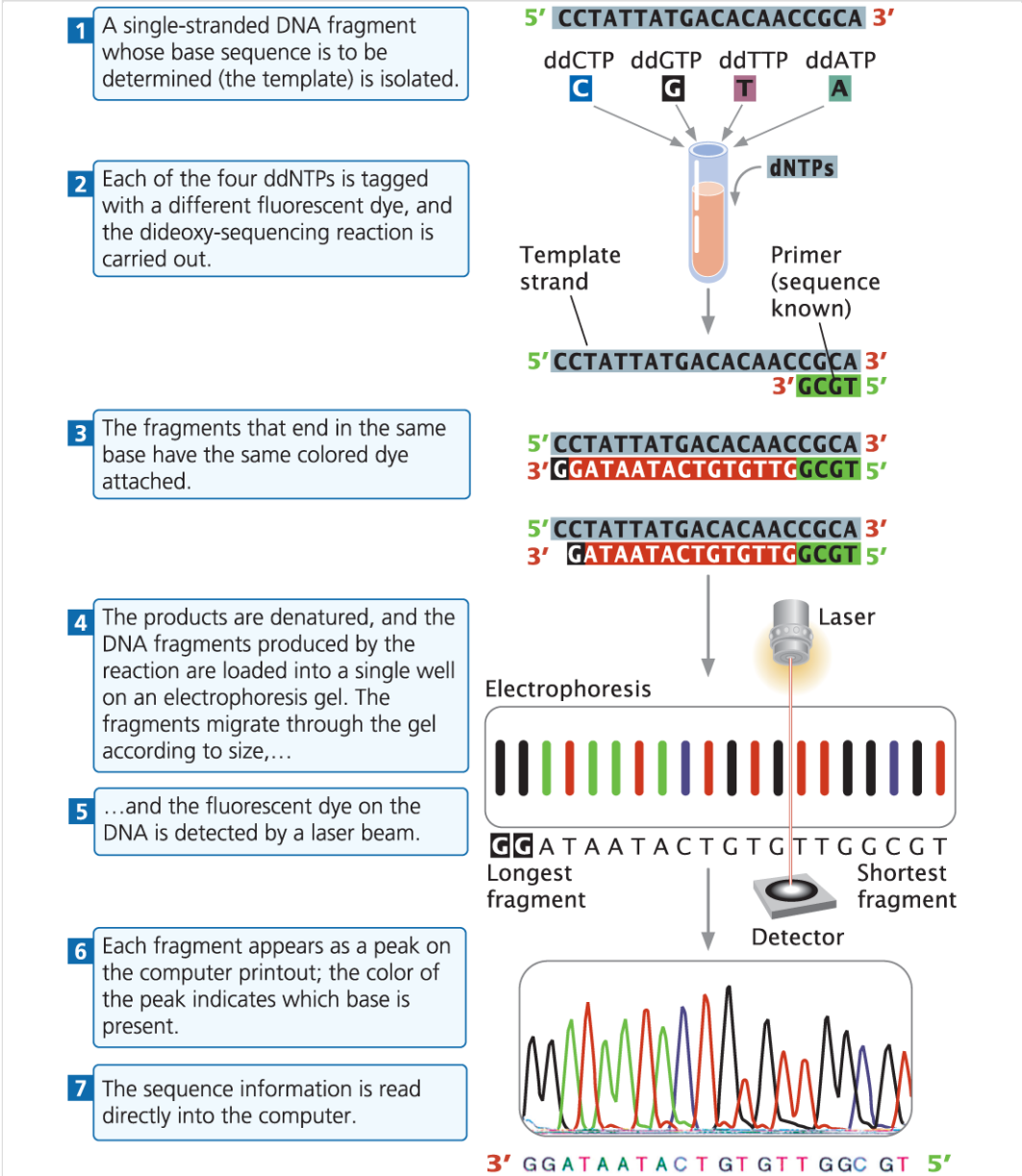
The contents of the four tubes are separated side by side on an acrylamide gel (which allows finer separation than agarose) so that DNA strands differing in length by only a single nucleotide can be distinguished. After electrophoresis, the locations, and therefore the sizes, of the DNA strands in the gel are revealed by the radioactive or chemical labels.

Reading the DNA sequence is the simplest and shortest part of the procedure. In Figure 19.17, you can see that the band closest to the bottom of the gel is from the tube that contained the ddGTP reaction, which means that the first nucleotide added was guanine

(G). The next band up is from the tube that contained ddATP, so the next nucleotide in the sequence is adenine (A), and so forth. In this way, the sequence is read from the bottom to the top of the gel, with the nucleotides near the bottom corresponding to the 5′ end of the newly synthesized DNA strand and those near the top corresponding to the 3′ end. Keep in mind that the sequence obtained is not that of the target DNA but that of its *complement*. To see dideoxy sequencing in action, view **Animation 19.4**. A **› TRY PROBLEM 46**

For many years, DNA sequencing was done largely by hand and was laborious and expensive. Today, sequencing is usually carried out by automated machines that use fluorescent dyes and laser scanners to sequence thousands of base pairs in a few hours (**Figure 19.18**). The dideoxy-sequencing reaction can also be used here, but the ddNTPs used in the reaction are labeled with fluorescent dyes, and a different colored dye is used for each type of ddNTP. In this case, the four sequencing reactions can take place in the same test tube and can be placed in the same well for electrophoresis. The sequencing devices carry out electrophoresis in gel-containing capillary tubes. The different-sized fragments produced by the sequencing reaction separate within a tube and migrate past a laser beam and detector. As the fragments pass the laser, their fluorescent dyes are activated. Each colored dye emits fluorescence of a characteristic wavelength, which is read by an optical scanner. The information is fed into a computer for interpretation, and the results are printed out as a set of peaks on a graph (see Figure 19.18). Automated sequencing

machines may contain 96 or more capillary tubes, allowing from 50,000 to 60,000 bp of sequence to be read in a few hours.



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19.18 The dideoxy-sequencing method can be automated.

Description

The illustration starts with a template strand with sequence: 5 prime-C C T A T T A T G A C A C A A C C G C A-3 prime. A caption reads, “A single-stranded D N A fragment whose base sequence is to be determined (the template) is isolated.” Four d d N T P’s namely; d d C T P, d d G T P, d d T T P, and d d A T P are added for each nucleotide in a test tube with d N T P’s. A caption reads, “Each of the four d d N T P’s is tagged with a different ¬fluorescent dye, and the dideoxy-sequencing reaction is carried out.” It leads to the template strand with the same sequence attached to a known primer sequence as follows: 3 prime-G C G T-5 prime. Then, a complementary strand forms. A caption reads, “The fragments that end in the same base have the same colored dye attached.” Then, the last nucleotide of the complementary strand, that is G, is eliminated from the strand. Electrophoretic bands are shown next with the use of laser. The sequence obtained is G G A T A A T A C T G T G T T G T C G T. The left side is labeled longest fragment with G G highlighted. The right side is labeled shortest fragment. A caption reads, “The products are denatured, and the D N A fragments produced by the reaction are loaded into a single well on an electrophoresis gel. The fragments migrate through the gel according to size, and the fluorescent dye on the D N A is detected by a laser beam.” A detector is then used to produce digital a graph with the help of laser. Two captions read as follows: “Each fragment appears as a peak on the computer printout; the color of the peak indicates which base is present. The sequence information is read directly into the computer.” The computer produced graph shows the following sequence: 3 prime-G G A T A A T A C T G T G T T G T C G T-5 prime.

CONCEPTS

The dideoxy-sequencing method uses ddNTPs, which terminate DNA synthesis at specific bases.

✔ CONCEPT CHECK 8

In the dideoxy-sequencing reaction, what terminates DNA synthesis at a particular base?

a. The absence of a base on the ddNTP halts the DNA polymerase.
b. The ddNTP causes a break in the sugar–phosphate backbone.
c. DNA polymerase will not incorporate a ddNTP into the growing DNA strand.

d. The absence of a 3′-OH group on the ddNTP prevents the addition of another nucleotide.

Next-Generation Sequencing Technologies

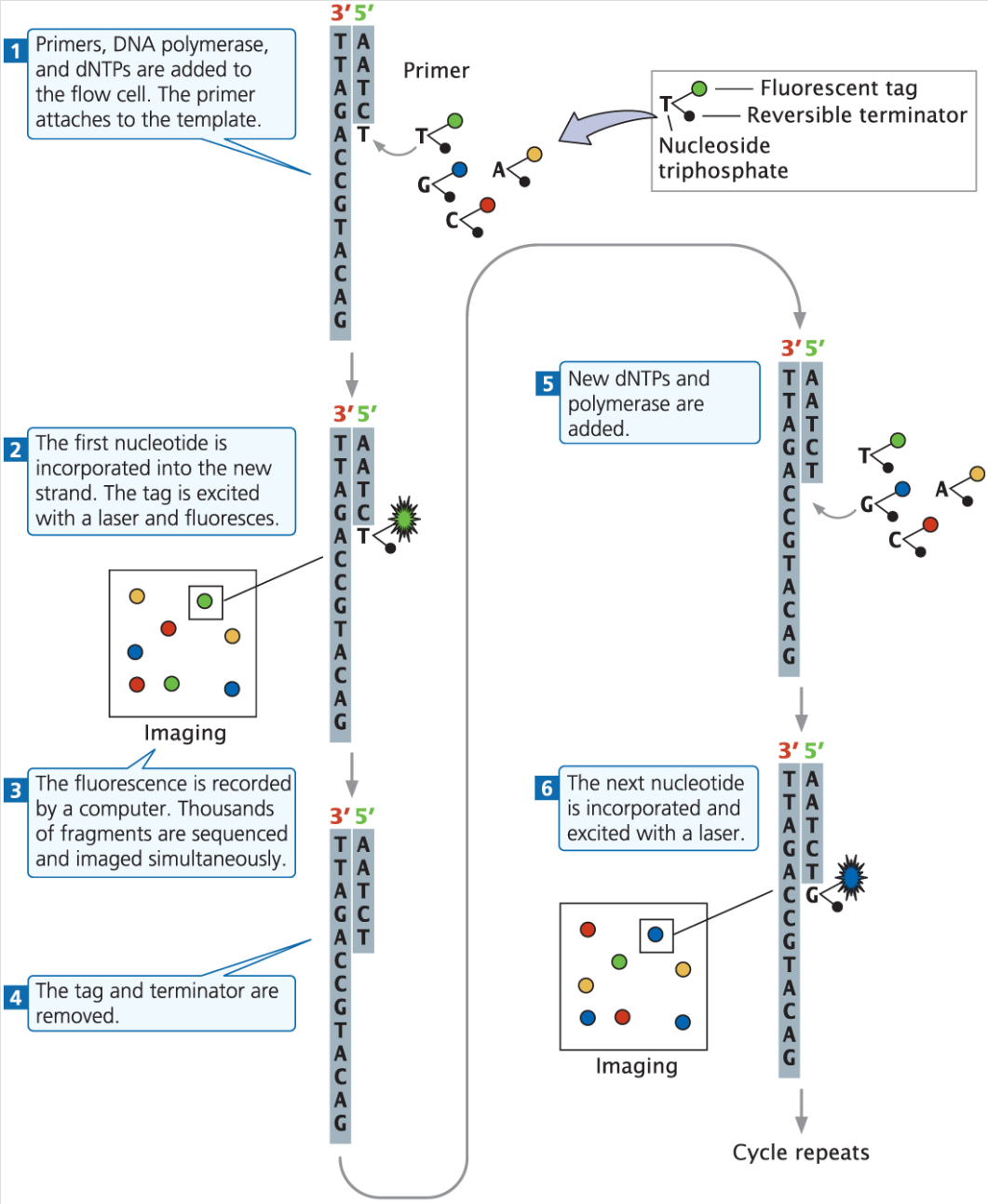
Newer methods, called **next-generation sequencing technologies**, have made sequencing hundreds of times faster and less expensive than the traditional Sanger sequencing method. Most next-generation sequencing technologies do sequencing in parallel, which means that hundreds of thousands or even millions of DNA fragments can be sequenced simultaneously, allowing, for example, a human genome to be sequenced in days instead of years.

ILLUMINA SEQUENCING

Illumina sequencing is the most widely used sequencing technology at the present time. It employs a technology that is similar in principle to that used in dideoxy sequencing. Special deoxynucleoside triphosphates (dNTPs) that have a fluorescent tag attached are used, with a differently colored tag for each type of dNTP. Each dNTP also has a chemical group (a terminator) that, once incorporated into the growing DNA chain, prevents the incorporation of any additional nucleotides. This is similar to

termination caused by dideoxyribonucleotides in Sanger sequencing. In this case, however, the terminator is reversible—it can be chemically removed.

To carry out sequencing, the target DNA is first cleaved into millions of short overlapping fragments. The fragments are attached to a surface of a reaction chamber called a flow cell and then amplified, creating clusters of up to 1000 copies of each fragment in close proximity on the surface. The fragments are then denatured, and a solution of primers, DNA polymerase, and the special nucleotides is added. The primer attaches to each DNA template, and the first nucleotide is incorporated into the newly synthesized strand (**Figure 19.19**). The solution is washed away, and the tag on the incorporated nucleotide is excited with a laser, which causes it to fluoresce. Each type of nucleotide (A, T, G, or C) has a differently colored fluorescent tag, so the color of the light produced reveals the type of nucleotide just added. The terminator and the fluorescent tag are then chemically removed, and the process is repeated. As the nucleotides are added one at a time, the sequence is read as a series of flashes of colored light from each cluster of DNA. Hundreds of thousands of DNA clusters, each consisting of copies of a different DNA fragment, are sequenced simultaneously, allowing large amounts of DNA to be sequenced in a short time.



19.19. Illumina sequencing is a next-generation sequencing technology.

Description

The illustration shows a template strand as follows: 3 prime-T T A G A C C G T A C A G-5 prime. A prime is attached with the following sequence: 5 prime-A A T C T-3 prime. d N T P's are added with fluorescent tag, reversible terminator, and nucleoside triphosphate. First d T T P is added. The tag is excited while imaging. Then, it shows a continuous

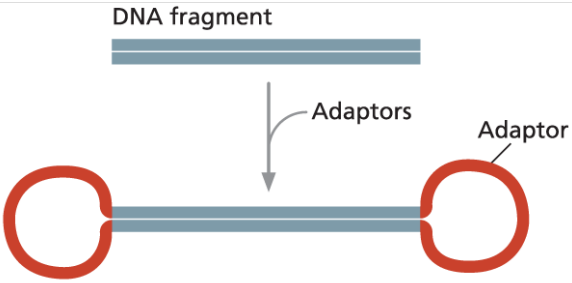
sequence of primer with the d T T P added. The same process continues with d G T P and the cycle repeats. The captions in the illustration read as follows: “Primers, D N A polymerase, and d N T P’s are added to the ¬flow cell. The primer attaches to the template. The first nucleotide is incorporated into the new strand. The tag is excited with a laser and fl¬uoresces. The fl¬uorescence is recorded by a computer. Thousands of fragments are sequenced and imaged simultaneously. The tag and terminator are removed. New d N T P’s and polymerase are added. The next nucleotide is incorporated and excited with a laser.”

THIRD-GENERATION SEQUENCING TECHNOLOGIES

Even more advanced sequencing methods, typically called third-generation sequencing technologies, are now being used. These technologies, also called single-molecule real-time (SMRT) sequencing, determine the sequence of single molecules of DNA or RNA and allow much longer fragments to be sequenced (produce longer reads), which simplifies the assembly of fragments into a complete genome (see Chapter 20). These technologies are called real-time sequencing because the methods do not require a pause between the addition of each nucleotide, as occurs in next-generation processes such as Illumina sequencing. Some third-generation sequencing technologies can also sequence types of DNA that are difficult to sequence with next-generation sequencing platforms, such as DNA with modified bases or with certain base compositions.

The third-generation sequencing method called PacBio—named after the company, Pacific Bio-Sciences, that helped develop and market

the technology—analyzes DNA synthesis of single molecules of DNA. To carry out PacBio sequencing, DNA is broken into fragments. Adaptors are attached to the ends of each fragment, creating a closed circle of DNA (**Figure 19.20**). The DNA molecules are then loaded onto a specially made chip, which contains over 100,000 very small sequencing chambers called zero-mode waveguides (ZMWs). Each ZMW holds the smallest volume in which light detection can occur. A single DNA molecule with the adaptors diffuses into a ZMW. Anchored at the bottom of the ZMW is a molecule of DNA polymerase, which binds to the adaptor at the end of the DNA circle and begins to carry out replication. The four types of dNTPs, each with a differently colored fluorescent label, are added to the chip. As a dNTP is held by the DNA polymerase within the ZMW before it is added to the newly synthesized DNA strand, the dNTP emits a pulse of light, the color of which reveals the type of base being added. The fluorescence label is cleaved off, and the next nucleotide is added. As synthesis proceeds, the pulses of light in the ZMWs are recorded by a computer, which translates the pulses into the base sequence of the fragment. Synthesis of different fragments is occurring simultaneously in thousands of ZMWs on a chip, so sequencing is rapid.



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19.20 In PacBio sequencing, adaptors are added to the end of each DNA fragment to produce a closed circle of DNA for sequencing.

Description

The illustration shows a D N A fragment represent by a straight horizontal line. A downward arrow shows the addition of adaptors. Two adaptors represented by circles are added at each end of the D N A fragment.

Because the template is circular, when the DNA polymerase reaches the end of the fragment, it proceeds around the adaptor and sequences the other strand. If the polymerase enzyme is active long enough, the same fragment can be sequenced multiple times, increasing the accuracy of the sequence record. Limitations of PacBio sequencing include higher error rates, a slower sequencing time, and a higher cost than next-generation sequencing methods. The biggest advantage is long read lengths. Average read lengths for PacBio sequencing are over 10,000 bp, and some are up to 60,000 bp. In comparison, maximum read lengths for Illumina sequencing are usually a few hundred base pairs.

Another third-generation sequencing technology is nanopore sequencing, which uses a completely different method. Here, a

single strand of DNA is passed through a tiny hole—a nanopore—in a membrane. As the molecule passes, one nucleotide at a time, through the nanopore, it disrupts an electrical current in the membrane, and the nature of the disruption is affected by the shape of the nucleotide passing through the nanopore. Each of the four bases of DNA causes a characteristic disruption, so the sequence of DNA can be read by analyzing the membrane current as the strand passes, one nucleotide at a time, through the nanopore. Hundreds of thousands of nanopores can be created on a single chip, so many DNA fragments can be read simultaneously. Like PacBio sequencing, nanopore sequencing allows for longer reads but has a much higher error rate.

In 2018, nanopore sequencing was used to sequence the entire genomes of 15 different *Drosophila* species at a cost of about $1,000 per genome. Because nanopore sequencing is based on electronics rather than chemistry, sequencing instruments can be packaged into very small, hand-held devices that are highly mobile. Learn more about next-generation and third-generation sequencing with **Animation 19.5**.

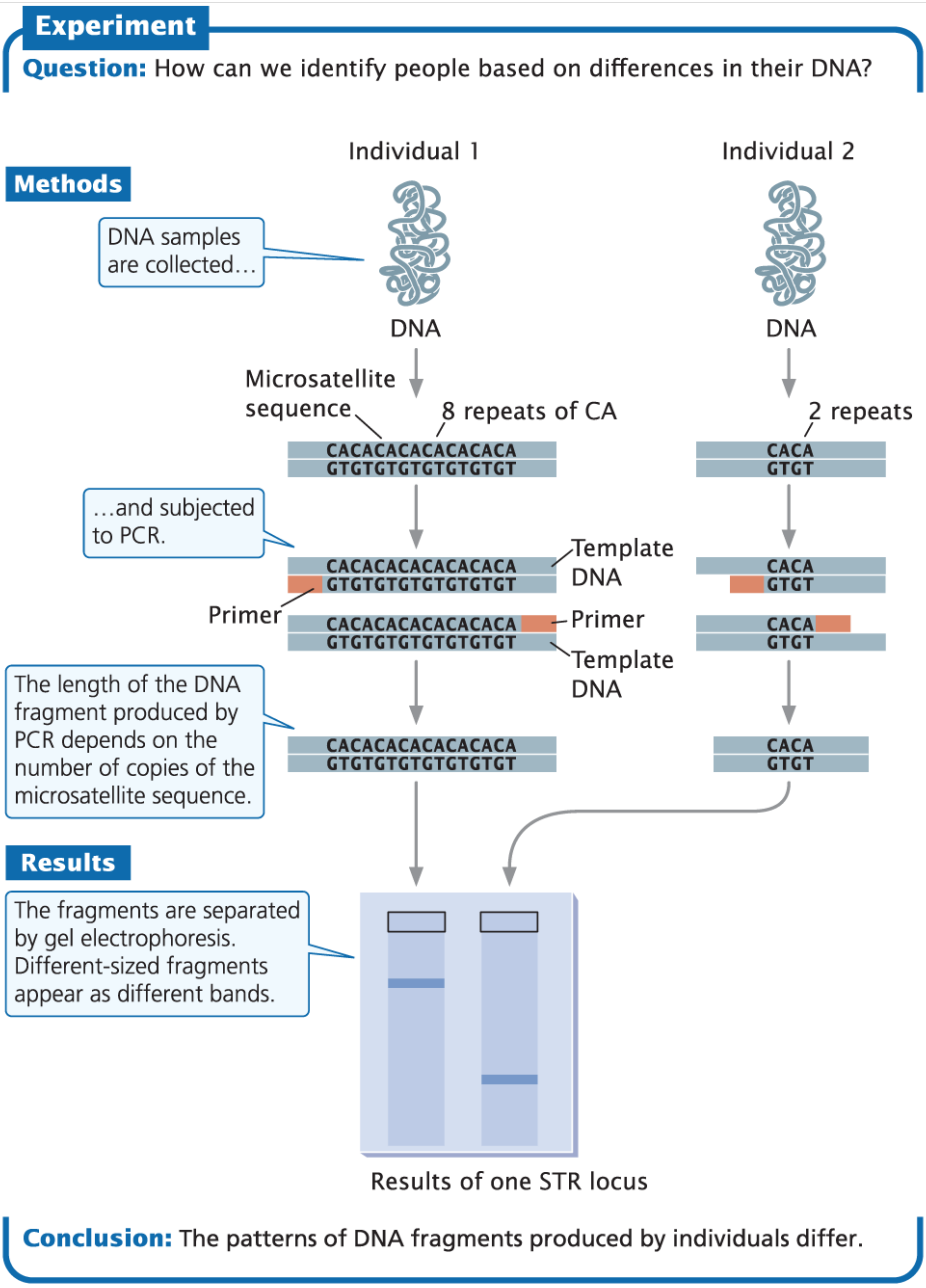
CONCEPTS

New next- and third-generation sequencing methods can sequence many DNA fragments simultaneously, providing a much faster and less expensive determination of a DNA base sequence than does the Sanger sequencing method.

DNA Fingerprinting

The use of DNA sequences to identify individual people is called **DNA fingerprinting** or *DNA profiling*. Because some parts of the genome are highly variable, each person's DNA sequence is unique and, like a traditional fingerprint, provides a distinctive characteristic that allows identification.

Most DNA fingerprinting uses **microsatellites**, or **short tandem repeats (STRs)**, which are very short DNA sequences repeated in tandem. These repeated sequences are found at many loci throughout the human genome. People vary in the number of copies of these repeats that they possess at each of these loci. The STRs are typically detected with PCR, using primers flanking the microsatellite repeats so that a DNA fragment containing the repeated sequences is amplified (**Figure 19.21**). The length of the amplified segment depends on the number of repeats; DNA from a person with more repeats will produce a longer amplified segment than will DNA from a person with fewer repeats.



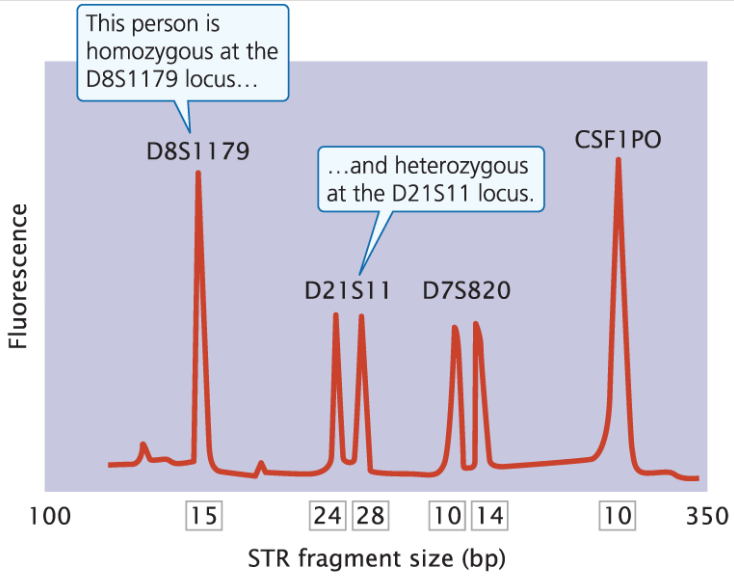
19.21 DNA fingerprinting can be used to identify people.

Description

The methods section shows the D N A loop of individual 1 on the left and on the right is the D N A loop of individual 2. Below this is a short sequence from their genomes containing microsatellite sequences. In individual 1, there are 8 repeats of sequence C-A, and in

individual 2, there are 2 repeats of C A. The D N A is collected and subjected to P C R. The length of the D N A fragment produced by P C R depends on the number of copies of the C-A sequence. The fragments are migrated through a gel. The gel is labeled results of one S T R locus. The results section shows a picture of the gel, in which, individual 1 has one large band, and individual 2 has one short band. The captions in the illustration are as follows: “D N A samples are collected and subjected to P C R. The length of the D N A fragment produced by P C R depends on the number of copies of the microsatellite sequence. The fragments are separated by gel electrophoresis. Different-sized fragments appear as different bands.”

In DNA fingerprinting, the primers used in PCR are tagged with fluorescent labels so that the resulting DNA fragments can be detected with a laser. Primers for different STR loci are given differently colored labels so that similar-sized products of different loci can be differentiated. After PCR, the fragments are separated on a gel or by a capillary electrophoresis machine, in which the presence of each fragment is detected as it migrates past a laser. A computer then calculates the size of each fragment based on its rate of migration. The fragments are represented as peaks on a graph; the distance on the horizontal axis represents the size of the fragment, while the height of the peak represents the amount of DNA (**Figure 19.22**). Homozygotes for an STR allele have a single tall peak; heterozygotes have two shorter peaks. When several different microsatellite loci are examined, the probability of two people having the same set of patterns becomes vanishingly small unless they are identical twins.



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19.22 A DNA profile represents the pattern of DNA fragments produced by performing PCR on the STR loci. This profile shows the results from four STR loci (D8S1179, D21S11, D7S820, and CSF1P0). The number below each peak represents the number of STRs in that DNA fragment.

Description

The horizontal axis represents S T R fragment size in base pairs between base pair lengths 100 and 350, while the vertical axis reads fluorescence. The profile shows 4 S T R loci as follows: It shows one peak for D 8 S 1 1 7 9, which is marked 15 at the horizontal axis and C S F 1 P O, which is marked 10 at the horizontal axis. A caption reads, “This person is homozygous at the D 8 S 1 1 7 9 locus and heterozygous at the D 2 1 S 1 1 locus.” The graph shows two peaks for D 2 1 S 1 1 marked 24 and 28 at the horizontal axis. The graph also shows two peaks D 7 S 8 2 0 marked 10 and 14 at the horizontal axis.

The Federal Bureau of Investigation has developed a system of STR loci (Table 19.4) that are used for identifying people and solving crimes. These loci make up the Combined DNA Index System (CODIS). Each STR locus in CODIS has a large number of alleles and

is located on a different human chromosome, so variation at each locus assorts independently. CODIS consists of 13 core STR loci that have been in use since 1990. In 2017, seven additional loci were added to increase the power of the system to discriminate among individuals and to make it compatible with international DNA databases.

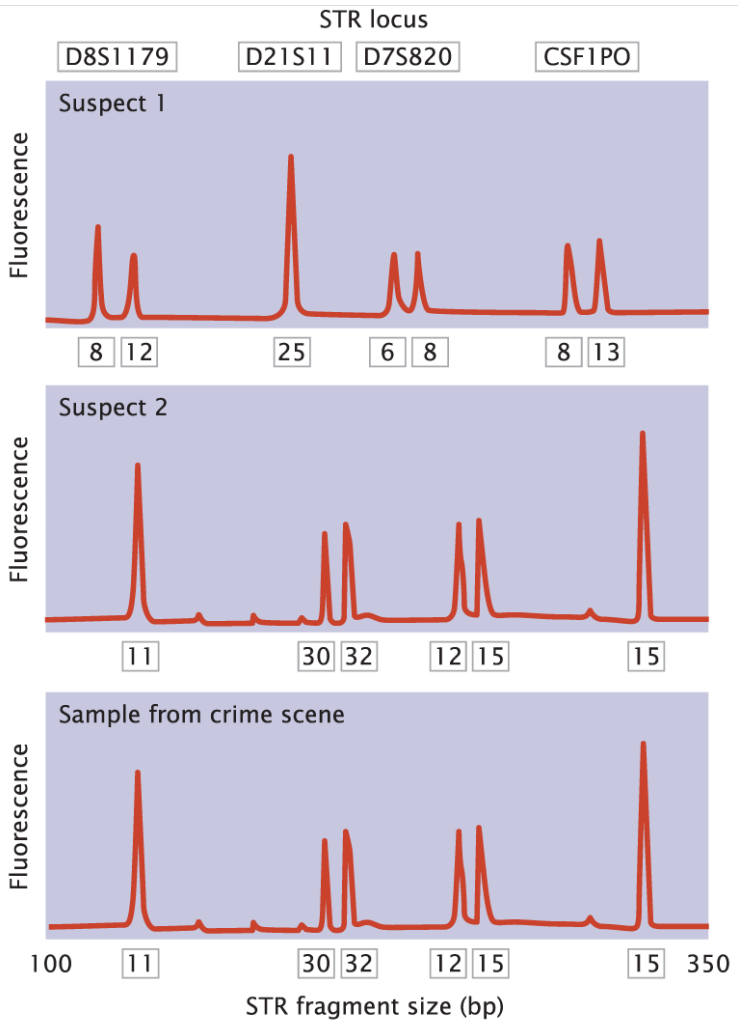
TABLE 19.4 Characteristics of the 13 original STR loci used in CODIS for DNA fingerprinting

Locus Name	Chromosome	Number of Repeats	Number of Alleles*
CSF1PO	5	5–17	10
FGA	4	12–51	23
TH01	11	3–14	8
TPOX	2	4–16	8
VWA	12	10–25	10
D3S1358	3	6–26	10
D5S818	5	4–29	9
D7S820	7	5–16	11
D8S1179	8	6–20	11
D13S317	13	5–17	8
D16S539	16	4–17	7
D18S51	18	5–40	19
D21S11	21	12–43	22

*US population.

Information from J. M. Butler and C. R. Hill, *Forensic Science Review* 24:15–26, 2012. In 2017, the following additional STR loci were added to CODIS: D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, and D22S1045.

In a typical application, DNA fingerprinting might be used to confirm that a suspect was present at the scene of a crime. A sample of DNA from blood, semen, hair, or some other body tissue is collected from the crime scene. If the sample is very small, PCR can be used to amplify it so that enough DNA is available for testing. Additional DNA samples are collected from one or more suspects. The pattern of DNA fragments produced by DNA fingerprinting of the sample is then compared with the pattern produced by DNA fingerprinting of the DNA from the suspect. A match between the sample and a suspect can provide evidence that the suspect was present at the scene of the crime (**Figure 19.23**).



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19.23 DNA fingerprinting can be used to determine the presence of a suspect at a crime scene. The DNA profile of suspect 2 matches that of DNA evidence collected at the crime scene. Shown here are results from four STR loci.

Description

The horizontal axis of each D N A profile represents S T R fragment size in base pairs between base pair lengths 100 and 350, while the vertical axis reads fluorescence. The three profiles show S T R locus for D 8 S 1179, D 21 S 11, D 7 S 820, and C S F 1 P O. Suspect 1 shows two peaks for D 8 S 1179 at 8 and 12, one peak for D 21 S 11 at 25, two peaks for D 7 S 820 at 6 and 8, and two peaks for C S F 1 P O at 8 and 13. Suspect 2 shows one peak for D 8 S 1179 at 11, two peaks for D 21 S 11 at 30 and 32, two peaks for D 7 S 820 at 12 and 15, and one peak for C S F 1 P O at 15. Sample from crime scene

shows one peak for D 8 S 1179 at 11, two peaks for D 21 S 11 at 30 and 32, two peaks for D 7 S 820 at 12 and 15, and one peak for C S F 1 P O at 15.

Since its introduction in the 1980s, DNA fingerprinting has helped convict a number of suspects in murder and rape cases. Suspects in other cases have been proved innocent when their DNA failed to match that from the crime scenes. Today, DNA fingerprinting has become widely accepted as an important tool in forensic investigations. In addition to its application in solving crimes, DNA fingerprinting is used to assess paternity, study genetic relationships among individual organisms in natural populations, identify specific strains of pathogenic bacteria, and identify human remains. Explore DNA fingerprinting in (A) **Animation 19.6.**

CONCEPTS

DNA fingerprinting detects genetic differences among individuals by analyzing highly variable regions of chromosomes.

✔ CONCEPT CHECK 9

How are microsatellites detected?

IDENTIFYING PEOPLE WHO DIED IN THE COLLAPSE OF THE WORLD TRADE CENTER WITH DNA FINGERPRINTING

On the morning of September 11, 2001, terrorists hijacked two passenger planes and flew them into the World Trade Center towers in New York City. The catastrophic damage and ensuing fire led to the complete collapse of both towers, killing almost 3000 building occupants and rescue personnel. The tremendous destructive force generated by the collapse of the towers pulverized many of the bodies beyond recognition.

In the days immediately following the World Trade Center collapse, forensic scientists began the task of identifying the remains of those who had perished. This task was unprecedented in scope and difficulty. In all, almost 20,000 individual remains were found, varying from whole bodies to tiny fragments of charred bone. The remains had been subjected to fires with temperatures exceeding 1000°C that burned for more than 3 months. The collapse of the buildings intermixed many victims' remains, and many body fragments were not recovered for months, during which time they were exposed to dust, water, bacteria, and decay. The usual means of victim identification—personal items, fingerprints, dental records—were of little use for most of the World Trade Center remains. Identification of the majority of the remains was made with the use of DNA fingerprinting (**Figure 19.24**).

Scott Gries/Getty Images.

19.24 DNA fingerprinting was used to identify the remains of people who died in the collapse of the World Trade Center. The New York City Office of Chief Medical Examiner used automated DNA fingerprinting to match DNA from human remains recovered from the World Trade Center with DNA extracted from reference samples, such as blood samples and toothbrushes, of possible victims.

DNA was first extracted from the tissue samples, and PCR was used to amplify STR loci in the CODIS system (see previous section). The DNA fingerprint generated from each sample was compared with that of DNA extracted from reference samples, such as victims' toothbrushes and blood samples. If the DNA from a tissue sample had the same alleles at all 13 loci as the reference sample, then a positive identification was made. When no reference sample was available, investigators collected DNA from victims' family members and tried to match the DNA profiles of remains to those of relatives; in this case, some, but not all, STR alleles would match.

For remains that were too badly degraded to amplify all STR loci, DNA fingerprinting was also carried out on mitochondrial DNA (see

Chapter 11). Because there are many mitochondria per cell and each mitochondrion contains multiple DNA molecules, there are many more copies of mitochondrial DNA per cell than nuclear DNA. Alone, fingerprinting from mitochondrial DNA was insufficient to provide identification with a high degree of confidence (because there are not as many sequences that vary among people as in the CODIS loci), but when it was used in conjunction with data from at least some STR loci, a positive identification could often be made. Used in combination, these techniques allowed the remains of over half of the victims to be positively identified.

19.6 Molecular Techniques Are Increasingly Used to Analyze Gene Function

In the preceding sections, we learned about the powerful molecular techniques that are available for amplifying, isolating, recombining, and sequencing DNA. Although these techniques provide a great deal of information about the organization and nature of gene sequences, the ultimate goal of many molecular studies is to better understand the function of these sequences. In this section, we explore some advanced molecular techniques that are frequently used to determine the functions of genes and to better understand the genetic processes that these sequences undergo.

Forward and Reverse Genetics

The traditional approach to the study of gene function begins with the identification of mutant organisms. For example, suppose a geneticist is interested in genes that affect cardiac function in mammals. A first step would be to find individuals—perhaps mice—that have hereditary defects in heart function. The mutations causing the cardiac problems in the mice could then be mapped, and the implicated genes could be isolated and sequenced. The proteins produced by the genes could then be predicted from the

gene sequences and isolated. Finally, the biochemistry of the proteins could be studied and their role in heart function discerned. This approach, which begins with a phenotype (a mutant individual) and proceeds to a gene that encodes the phenotype, is called **forward genetics**.

An alternative approach is to begin with a genotype—a DNA sequence—and proceed to the phenotype by altering the sequence or inhibiting its expression. A geneticist might begin with a gene of unknown function, induce mutations in it, and then observe the effect of these mutations on the phenotype of the organism. This approach is called **reverse genetics**. Today, both forward and reverse genetic approaches are widely used in analyses of gene function.

Creating Random Mutations

Forward genetics depends on the identification and isolation of random mutations that affect a phenotype of interest. Early in the study of genetics, geneticists were forced to rely on naturally occurring mutations, which are usually rare and can be detected only if large numbers of organisms are examined. The discovery of mutagens—environmental factors that increase the rate of mutation (see Section 18.2)—provided a means of increasing the number of mutants in experimental populations of organisms. One of the first examples of experimentally created mutations was Hermann

Muller's use of X-rays in 1927 to induce X-linked mutations in *Drosophila melanogaster*.

Today, radiation, chemical mutagens, and transposable elements are all used to create mutations for genetic analysis. To determine all genes that might affect a phenotype, it is desirable to create mutations in as many genes as possible—that is, to saturate the genome with mutations. This procedure, known as a mutagenesis screen, will be described in Chapter 20.

Targeted Mutagenesis

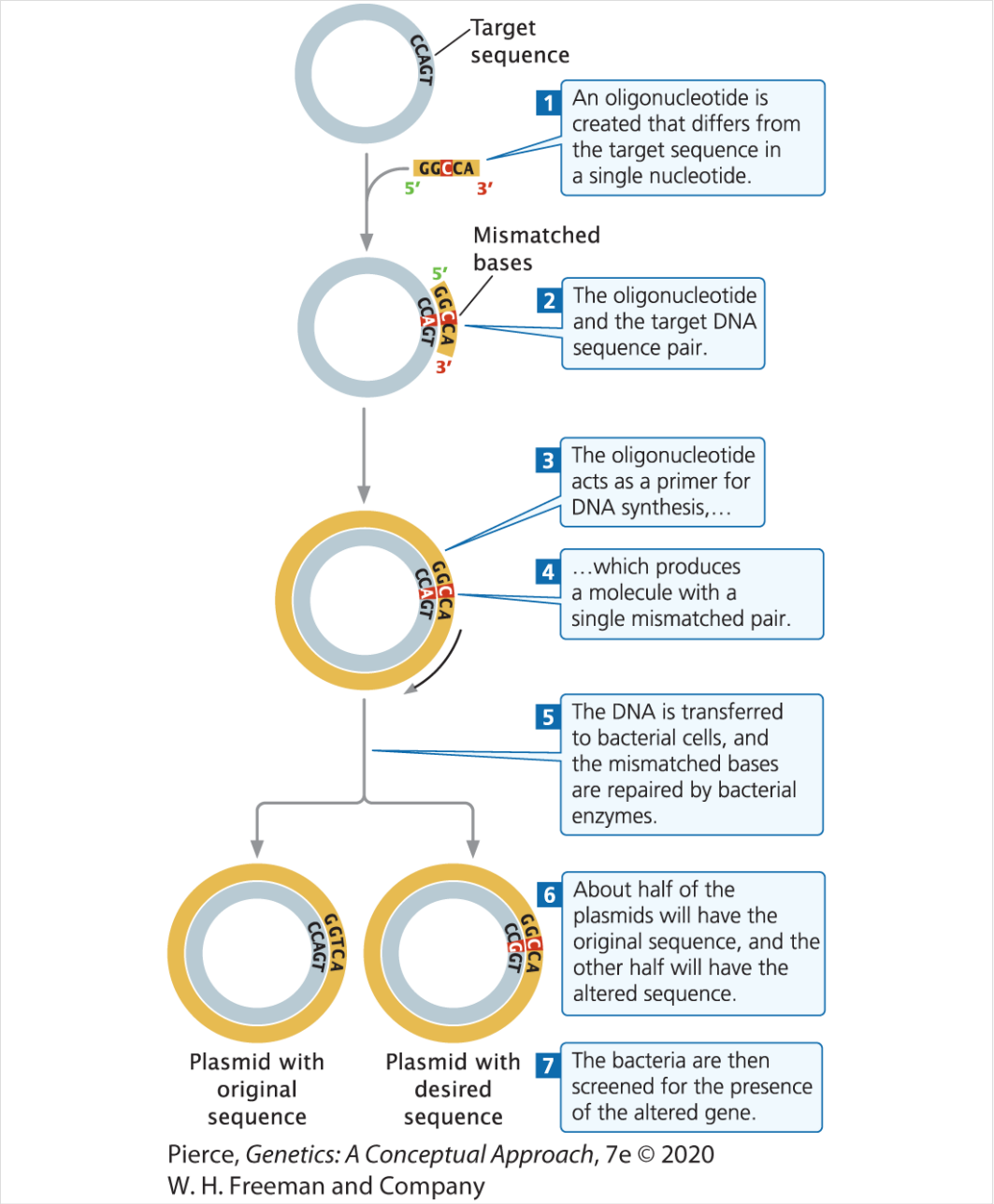
Reverse genetics depends on the ability to create mutations not at random but in particular DNA sequences, and then to study the effects of these mutations on the organism. Mutations are induced at specific locations through a process called **targeted mutagenesis**.

A number of different strategies have been developed for targeted mutagenesis. The CRISPR-Cas9 system (see Section 19.2) can be used to create mutations at specific sites. Single guide RNAs are designed to be complementary to a target sequence. The sgRNA and Cas9 are inserted into a cell, where Cas9 is guided to the target site by the sgRNA and makes double-stranded cuts at the site. Short duplications and deletions are often introduced when the cuts are repaired by nonhomologous end joining. Alternatively, donor DNA containing a specific desired mutation can be provided along with

CRISPR-Cas9; the donor DNA will be inserted at the target site by homology directed repair.

Another strategy, called **site-directed mutagenesis**, is to cut out a short sequence of nucleotides with restriction enzymes and replace it with a synthetic oligonucleotide—a short DNA fragment that contains the desired mutated sequence. The success of this method depends on the availability of restriction sites flanking the sequence to be altered.

If appropriate restriction sites are not available, **oligonucleotide-directed mutagenesis** can be used (**Figure 19.25**). In this method, a single-stranded oligonucleotide is produced that differs from the target sequence by one or a few bases. Because they differ in only a few bases, the target DNA and the oligonucleotide will pair. When successfully paired with the target DNA, the oligonucleotide can act as a primer to initiate DNA synthesis, which produces a double-stranded molecule with a mismatch in the primer region. When this DNA is transferred to bacterial cells, the mismatched bases will be repaired by bacterial enzymes. About half of the time, the normal bases will be changed into mutant bases, and about half of the time, the mutant bases will be changed into normal bases. The bacteria are then screened for the presence of the mutant gene.



19.25 Oligonucleotide-directed mutagenesis is used to study gene function when appropriate restriction sites are not available.

Description

The flowchart starts with a circular plasmid having target sequence as C C A G T. An oligonucleotide is added with the sequence 5 prime-G G C C A-3 prime. A caption reads, “An oligonucleotide is created that differs from the target sequence in a single nucleotide.” The oligonucleotide pairs with the target sequence on plasmid and it is labeled

“mismatched bases”. A caption reads, “The oligonucleotide and the target D N A sequence pair.” The mismatched base pair, C and A is shown. A newly synthesized plasmid is shown over the template plasmid. A caption reads, “The oligonucleotide acts as a primer for D N A synthesis, which produces a molecule with a single mismatched pair.” The plasmid then shows two branches having a double-stranded plasmid each. One plasmid is with original sequence that is C C A G T with its complementary strand having G G T C A. The other plasmid is with the desired sequence that is C C G G T with its complementary strand having G G C C A. Another caption reads, “About half of the plasmids will have the original sequence, and the other half will have the altered sequence. The bacteria are then screened for the presence of the altered gene.”

CONCEPTS

Forward genetics begins with a phenotype and detects and analyzes the genotype that causes that phenotype. Reverse genetics begins with a gene sequence and determines the phenotype it encodes. Particular mutations can be introduced at specific sites within a gene by means of CRISPR-Cas editing and site-directed and oligonucleotide-directed mutagenesis.

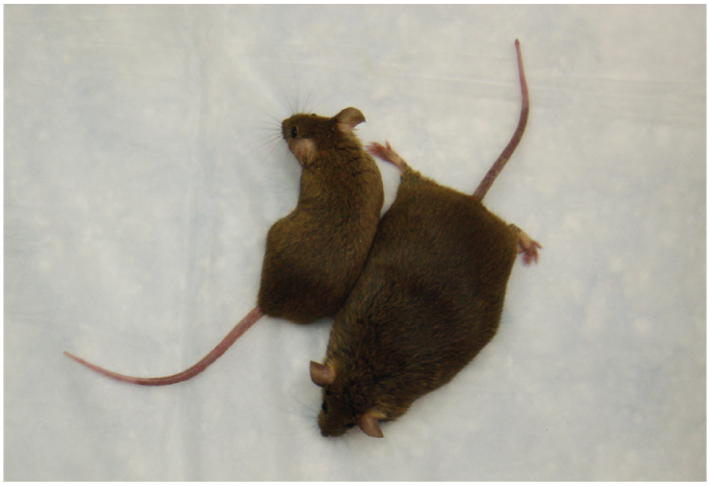
✔ CONCEPT CHECK 10

A geneticist interested in immune function induces random mutations in a number of specific genes in mice and then determines which of the resulting mutant mice have impaired immune function. This procedure is an example of

a. forward genetics.
b. reverse genetics.
c. both forward and reverse genetics.
d. neither forward nor reverse genetics.

Transgenic Animals

Another way in which gene function can be analyzed is by adding DNA sequences of interest to the genome of an organism that normally lacks such sequences and then observing the effect of the introduced sequences on the organism's phenotype. This method is a form of reverse genetics. An organism that has been permanently altered by the addition of a DNA sequence to its genome is said to be *transgenic*, and the foreign DNA that it carries is called a **transgene** (**Figure 19.26**). Here we consider techniques for the creation of transgenic mice, which are often used in the study of the function of human genes because they can be genetically manipulated in ways that are impossible with humans and because, as mammals, they are more similar to humans than are fruit flies, fish, and other model genetic organisms.



Dr. Liangyou Rui, Professor, Molecular & Integrative Physiology, University of Michigan.

19.26 The genome of a transgenic organism has been permanently altered by genetic engineering. The mouse on the right is transgenic for a mutation that causes obesity.

The oocytes of mice and other mammals are large enough that DNA can be injected into them directly. Immediately after penetration by a sperm, a fertilized mouse egg contains two pronuclei, one from the sperm and one from the egg; these pronuclei later fuse to form the nucleus of the embryo. Mechanical devices can manipulate extremely fine, hollow glass needles to inject DNA directly into one of the pronuclei of a fertilized egg (**Figure 19.27**). Typically, a few hundred copies of cloned, linear DNA are injected into a pronucleus. In a few of the injected eggs, copies of the cloned DNA integrate randomly into one of the chromosomes through a process called nonhomologous recombination. Today, researchers often use CRISPR-Cas, injecting one or more sgRNAs, along with mRNA for Cas9, into mice embryos (see Section 19.2). The Cas9 enzyme is produced within the embryo, combines with the sgRNA, and is targeted to a specific DNA sequence, where it carries out precise cleavage of the DNA. Nonhomologous end joining produces insertions and deletions in the edited DNA, producing knockouts (see next section). Alternatively, a piece of double-stranded DNA with a specific DNA sequence can also be injected along with the sgRNA and Cas9 mRNA; homology directed repair may insert the donor DNA sequence into the break. Gene editing has also been accomplished by using viruses to carry the CRISPR-Cas9 components into mouse embryos instead of injecting them.

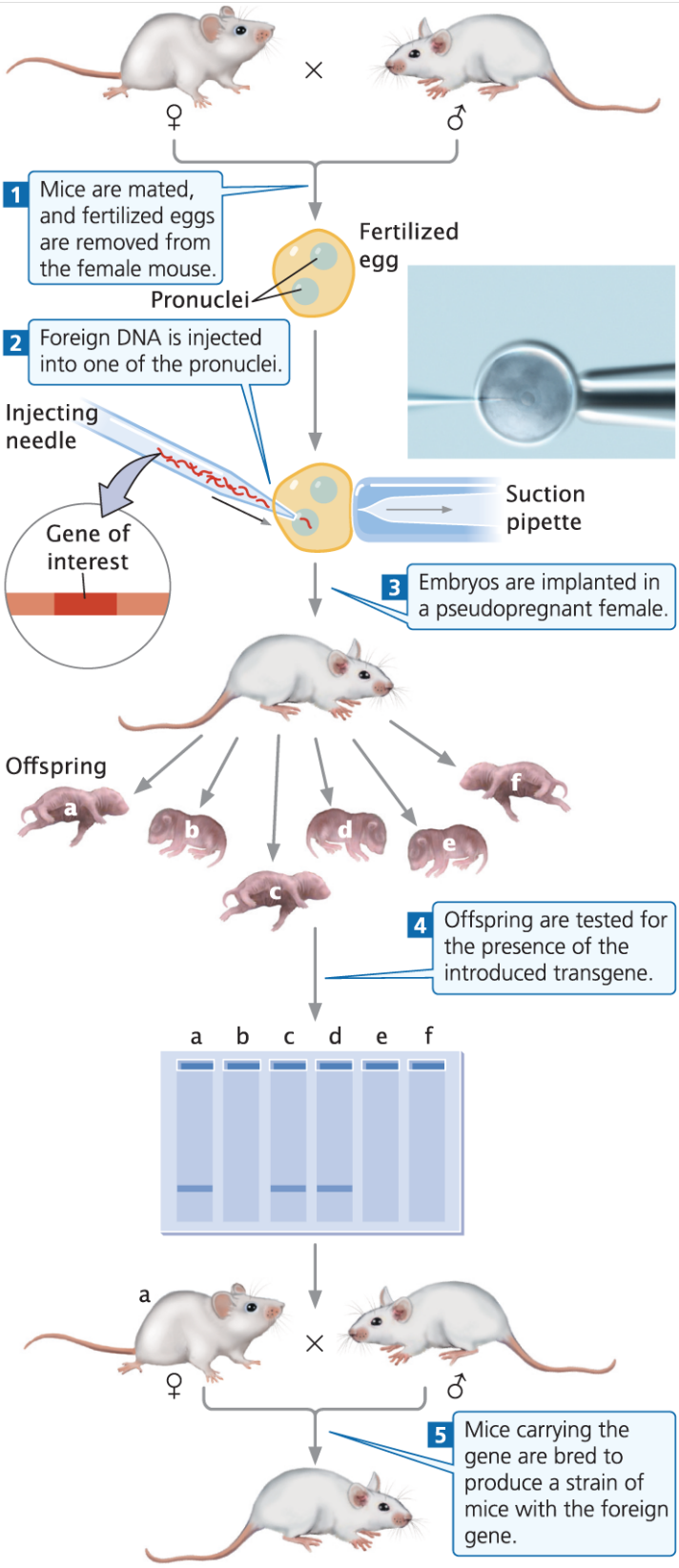


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Photo: Chad Baker/Thomas Northcut/Getty Images.

19.27 Transgenic animals have genomes that have been permanently altered through recombinant DNA technology. In the photograph, a mouse embryo is being injected with DNA.

Description
The illustration starts with a cross between a male and a female mouse, which leads to a fertilized egg having two pronuclei. A caption reads, “Mice are mated, and fertilized eggs are removed from the female mouse. Foreign D N A is injected into one of the pronuclei.” An injecting needle is used to inject the gene of interest into one pronucleus. A suction pipette is shown on one end of the egg. A caption reads, “Embryos are implanted in a pseudopregnant female.” This egg gives rise to a mouse, which further produces six offspring labeled “a” through “f”. A caption reads, “Offspring are tested for the presence of the introduced transgene.” Bands are shown for all these offspring through gel electrophoresis. One female and one male mouse are crossed again; one of them is labeled “a”, which further gives rise to a mouse. A caption reads, “Mice carrying the gene are bred to produce a strain of mice with the foreign gene.”

After injection, the embryos are implanted in a pseudopregnant female: a surrogate mother that has been physiologically prepared for pregnancy by mating with a vasectomized male.

Only about 10% to 30% of the embryos survive, and of those that do survive, only some carry a copy of the cloned DNA stably integrated into a chromosome. Nevertheless, if several hundred embryos are injected and implanted, there is a good chance that one or more mice whose chromosomes contain the foreign DNA will be born. Moreover, because the DNA was injected at the one-cell stage of the embryo, these mice usually carry the cloned DNA in every cell of their bodies, including their reproductive cells, and will therefore

pass the foreign DNA on to their progeny. Through interbreeding, a strain of mice that carry the foreign gene can be created.

Transgenic mice have proven useful in the study of gene function. For example, proof that the *SRY* gene (see Chapter 4) is the male-determining gene in mice was obtained by injecting a copy of the *SRY* gene into XX embryos and observing that these mice developed as males. In addition, researchers have created a number of transgenic mouse strains that serve as experimental models for human genetic diseases.

Knockout Mice

A useful variant of the transgenic approach is to produce mice in which a normal gene has been not just mutated but fully disabled. These animals, called **knockout mice**, are particularly helpful in determining the function of a gene: the phenotype of the knockout mouse often gives a good indication of the function of the missing gene.

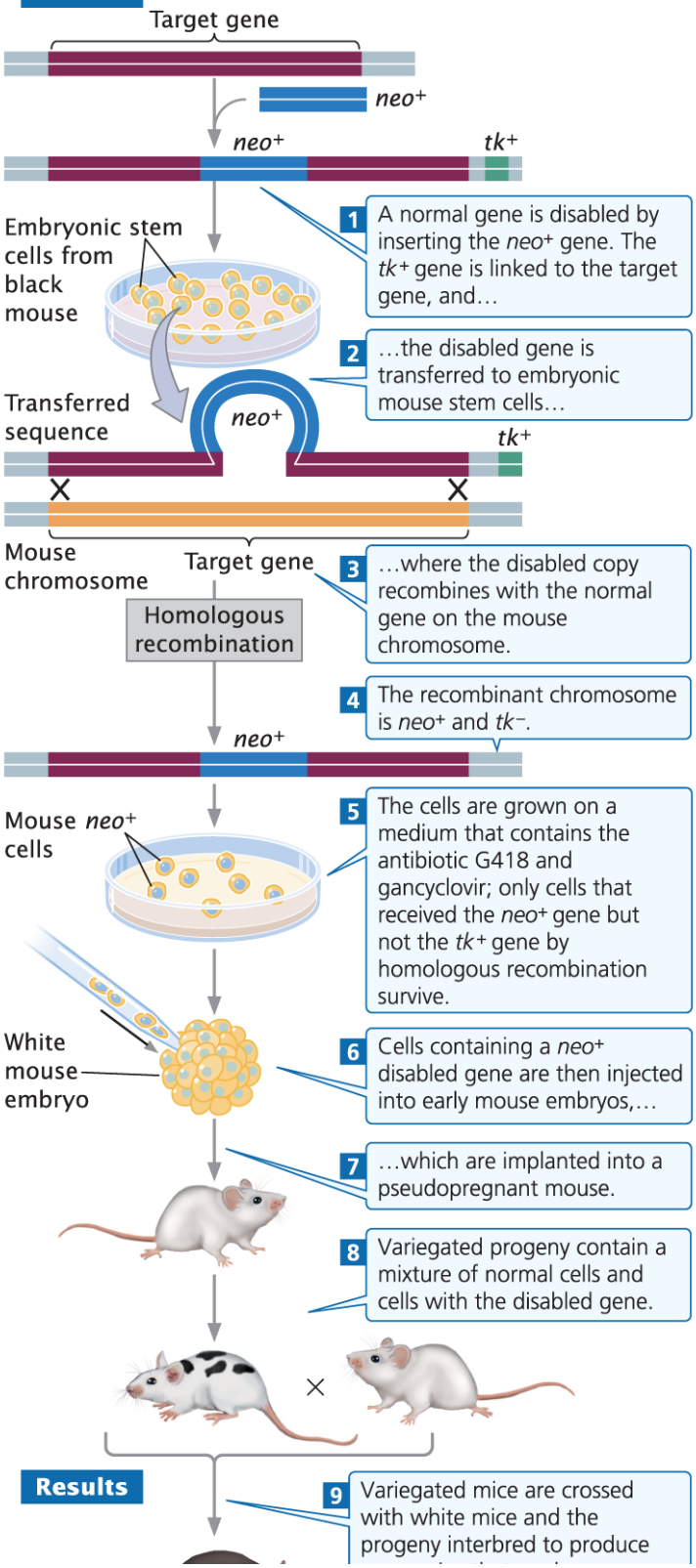
Today, knockout mice are often created by using CRISPR-Cas technology (see previous section). Originally, the creation of knockout mice was carried out by cloning a normal gene in bacteria and then "knocking it out," or disabling it. There are a number of ways to disable a gene, but a common method is to insert a gene called *neo*, which confers resistance to the antibiotic G418, into the

middle of the target gene (**Figure 19.28**). The insertion of *neo* both disrupts the target gene and provides a convenient marker for finding copies of the disabled gene. In addition, a second gene, usually the herpes simplex viral thymidine kinase (*tk*) gene, is linked to the disabled gene. The disabled gene is then transferred to cultured mouse embryonic cells, where it may exchange places with the normal copy on the mouse chromosome through homologous recombination.

Experiment

Question: How can the function of a gene be determined?

Methods



Results

9 Variegated mice are crossed with white mice and the progeny interbred to produce

some mice that are homozygous for the knocked-out gene.

Conclusion: Through this procedure, mice that contain no functional copy of the gene—that is, the gene is knocked out—are produced. The phenotype of the knockout mice reveals the function of the gene.

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19.28 Knockout mice possess a genome in which a gene has been disabled.

Description

The experiment starts with a question: “How can the function of a gene be determined?” The Methods section shows a D N A possessing a target gene. Neo superscript positive gene is added in between the target sequence and t k superscript positive gene is added at one end. A caption reads, “A normal gene is disabled by inserting the neo superscript positive gene. The t k superscript positive gene is linked to the target gene, and the disabled gene is transferred to embryonic mouse stem cells where the disabled copy recombines with the normal gene on the mouse chromosome. The recombinant chromosome is neo superscript positive and t k superscript negative.” This leads to a petri dish having embryonic stem cells from a black mouse. An arrow from these cells shows a D N A labeled transferred sequence. It shows D N A similar to the earlier D N A, but with a curve on the neo superscript positive gene. It recombines with mouse chromosome having the target gene. Homologous recombination takes place. The chromosome now lacks the t k superscript positive gene. Mouse neo superscript positive cells are grown from it in a petri dish. A caption reads, “The cells are grown on a medium that contains the antibiotic G 418 and gancyclovir; only cells that received the neo superscript positive gene but not the t k superscript positive gene by homologous recombination survive.” The illustration then shows white mouse embryos being injected with a needle. A downward arrow leads to a mouse. A caption reads, “Cells containing a neo superscript positive disabled gene are then injected into early mouse embryos, which are implanted into a pseudopregnant mouse.” This mouse gives rise to a mouse with black spots. A caption reads, “Variegated progeny contain a mixture of normal cells and cells with the disabled gene.” It is crossed with a white mouse and produces a black mouse. A caption reads, “Variegated mice are crossed with white mice and the progeny interbred to produce some mice that are homozygous for the knocked-out gene.” Text at the bottom reads, “Conclusion: Through this procedure, mice that contain no functional copy of the gene—that is, the gene is

knocked out—are produced. The phenotype of the knockout mice reveals the function of the gene."

After the disabled gene has been transferred to the embryonic cells, the cells are screened by adding the antibiotic G418 to their growth medium. Only cells with the disabled gene containing the *neo* insert will survive. Because the frequency of nonhomologous recombination is higher than that of homologous recombination and because the intact target gene is replaced by the disabled copy only through homologous recombination, a means to select for the rarer homologous recombinants is required. The presence of the viral *tk* gene makes the cells sensitive to the antimicrobial gancyclovir. Thus, transfected cells that grow on a medium containing G418 and gancyclovir contain the *neo* gene (and thus the disabled target gene) but not the adjacent *tk* gene, because the *tk* gene has been eliminated in the double-crossover event. These cells contain the desired homologous recombinants. The nonhomologous recombinants (random insertions) contain both the *neo* and the *tk* genes, and these transfected cells die on the selection medium owing to the presence of gancyclovir. The surviving cells are injected into an early-stage mouse embryo, which is then implanted into a pseudopregnant mouse. Cells in the embryo carrying the disabled gene and normal embryonic cells carrying the wild-type gene will develop together, producing a chimera—a mouse that is a genetic mixture of the two cell types. The production of chimeric mice is not itself desirable, but replacing all the cells of the embryo with injected cells is impossible.

Chimeric mice can be easily identified if the injected embryonic cells came from a black mouse and the embryos into which they are injected came from a white mouse; the resulting chimeras will have variegated black and white fur. Some of the chimeras may have the knockout gene in their germ-line cells and can transmit it to the next generation. The chimeras are crossed to white mice; any black progeny are heterozygous for the knockout. The black progeny can then be intercrossed to produce some progeny that are homozygous for the knockout gene. The effects of disabling a particular gene can be observed in these homozygous mice. Three scientists who helped develop the original techniques for creating knockout mice—Mario Capecchi, Oliver Smithies, and Martin Evans—were awarded the Nobel Prize in physiology or medicine in 2007 for their work.

Additional techniques have been developed for knocking out genes only in certain tissues or at certain times; the resulting mice are called conditional knockouts. A variant of the knockout procedure is to insert a particular DNA sequence at a known chromosome location. For example, researchers might insert the sequence of a human disease-causing allele into the same locus in mice, creating a precise mouse model of the human disease. Mice that carry inserted sequences at specific locations are called **knock-in mice**. See how transgeneic animals are created in (A) **Animation 19.7**.

CONCEPTS

A transgenic mouse is produced by injecting cloned DNA into the pronucleus of a fertilized egg and then implanting the egg in a female mouse. In knockout mice, mutations that disable a gene are added or induced.

✔ CONCEPT CHECK 11

What is the advantage of using the *neo* gene to disrupt the function of a gene in knockout mice?

a. The *neo* gene produces an antibiotic that kills unwanted cells.
b. The *neo* gene is the right size for disabling other genes.
c. The *neo* gene provides a selectable marker for finding cells that contain the disabled gene.
d. The *neo* gene produces a toxin that inhibits transcription of the target gene.

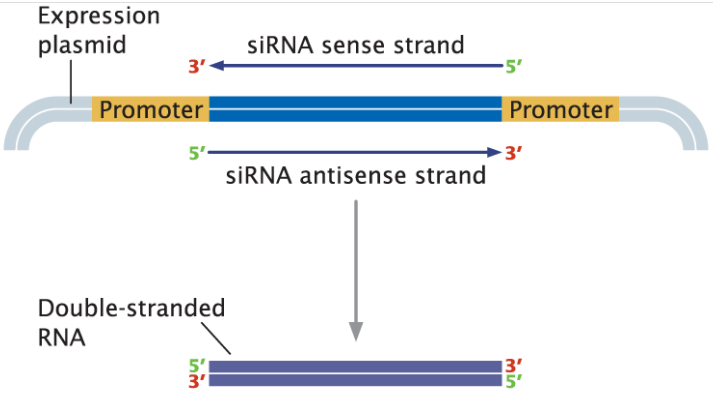
Silencing Genes with RNAi

In the preceding sections, we have seen that introducing mutations or new DNA sequences into the genome and observing the resulting phenotypes can provide information about the function of the altered or introduced DNA. We can also analyze gene function by temporarily turning a gene off and observing the effect of the absence of the gene product on the phenotype. For many years, there was no such method for selectively affecting gene expression. However, the discovery of siRNAs (small interfering RNAs) and miRNAs (microRNAs; see Chapters 14 and 17) has provided powerful tools for controlling the expression of individual genes.

Recall that siRNAs are small RNA molecules that combine with proteins to form an RNA-induced silencing complex (RISC). In a process called RNA interference, or RNAi, the RISC pairs with complementary sequences on mRNA and either cleaves the mRNA or prevents it from being translated. Molecular geneticists have exploited this natural machinery for turning off the expression of specific genes. The effect of silencing a gene with siRNAs can often be a source of insight into the gene's function.

The first step in using RNAi technology is to design siRNAs that will be recognized and cleaved by Dicer (the protein that processes siRNAs; see Section 14.5). The complementary sequence must be unique to the target mRNA and must not be found on other mRNAs, so that the siRNA will not inhibit nontarget mRNAs. Computer programs are often used to design optimal siRNAs.

After the siRNA sequence has been designed, it must be synthesized. One way to create an siRNA is to use an oligonucleotide synthesizer to synthesize a DNA fragment corresponding to the siRNA sequence. The synthesized oligonucleotide can be cloned into a plasmid expression vector between two strong promoters (**Figure 19.29**). *Escherichia coli* are then transformed with the plasmid. Within the bacteria, transcription from the two promoters will proceed in both directions, producing two complementary RNA molecules that will pair to form a double-stranded RNA molecule recognized by Dicer. Alternatively, double-stranded RNA sequences can be synthesized directly.



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19.29 Small interfering RNAs can be produced by cloning DNA sequences corresponding to the siRNAs between two strong promoters. When cloned into an expression vector, both DNA strands will be transcribed, and the complementary RNA molecules will anneal to form double-stranded RNA that will be processed into siRNA by Dicer.

Description
The two strands are located between two promoters and lead to the formation of a double-stranded R N A.

The next task is to deliver the double-stranded siRNA to the target cells. Delivery can be accomplished in a variety of ways, depending on the cell type. The model genetic organism *Caenorhabditis elegans* (see p. A6) can be fed *E. coli* (their natural food) containing the expression vector. Transcription within the bacteria produces double-stranded RNA, which the worms ingest and incorporate into their cells. Alternatively, double-stranded siRNA can be injected directly into the cells or the body cavity. Yet another approach is to synthesize a short sequence of DNA that has internal complementarity so that, when transcribed, its RNA product folds

up into a short hairpin RNA (shRNA) with a double-stranded section. Within a cell, the shRNAs are processed by Dicer to produce siRNAs that bring about gene silencing. DNA sequences containing siRNAs can be introduced into a vector using standard cloning techniques, and the vector can be used to deliver the DNA to a cell. An advantage of this approach is that with the addition of a DNA sequence, the RNAi sequence has the potential to become a permanent part of the cell's genome and be passed on to progeny.

One of the major advantages of siRNA for controlling gene expression is that it acts in trans—that is, a single copy of an siRNA gene will shut down expression of both copies of the target gene. Another advantage is that the target gene remains intact, and therefore the silencing effects are reversible. › **TRY** PROBLEM 52

Using RNAi to Treat Human Disease

In addition to its value in determining gene function, RNAi holds potential as a therapeutic agent for the treatment of human diseases.

TREATMENT OF HIGH CHOLESTEROL WITH RNAi

Research has examined the potential of RNAi for the treatment of cholesterol metabolism disorders. Although cholesterol is essential

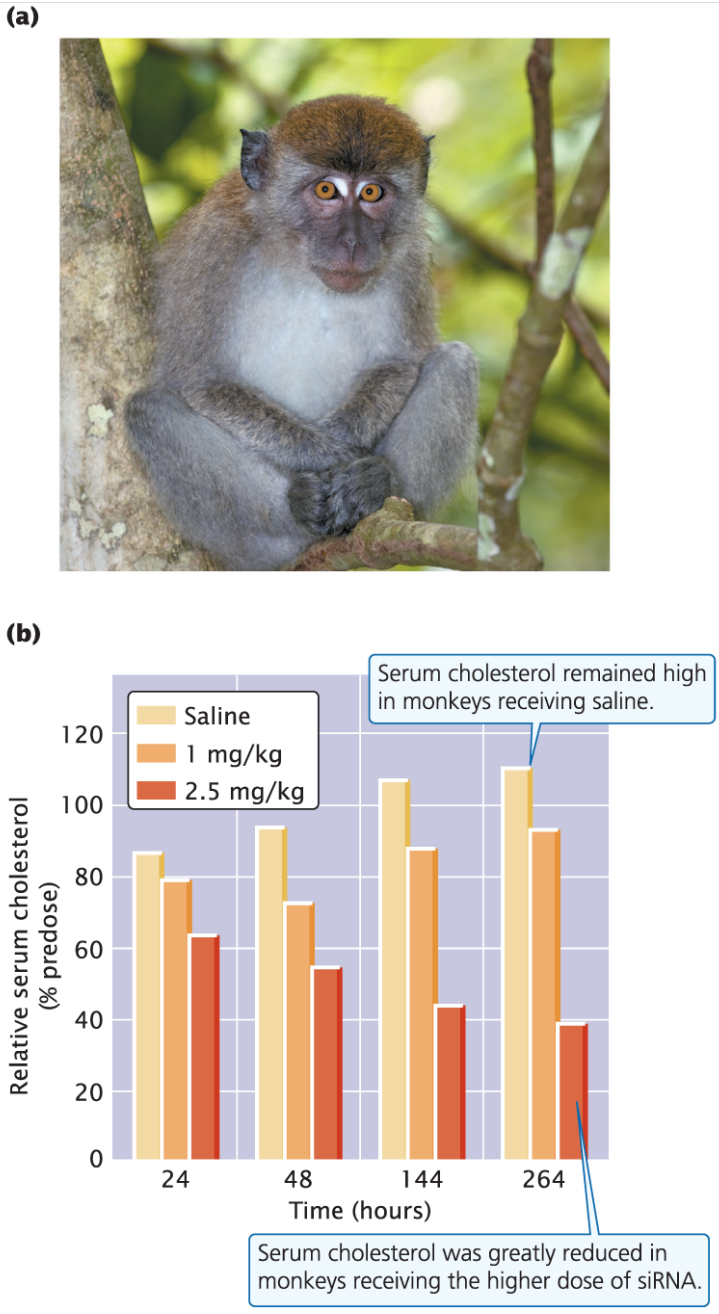
for life, too much cholesterol is unhealthy: high blood cholesterol is a major contributor to heart disease, the leading cause of death in the United States. Cholesterol is normally transported throughout the body in the form of small particles called lipoproteins, which consist of a core of lipids surrounded by a shell of phospholipids and proteins (see Figure 6.6). The ApoB protein is an essential part of lipoproteins. Some people possess genetic mutations that cause elevated levels of ApoB, which predispose them to coronary artery disease. Findings from studies suggest that lowering the amount of ApoB can reduce the number of lipoproteins and lower blood cholesterol in these people, as well as in people who have elevated cholesterol for other reasons.

In 2006, Tracy Zimmermann and her colleagues at Alnylam Pharmaceuticals and Protiva Biotherapeutics demonstrated that RNAi could be used to reduce the levels of ApoB and blood cholesterol in nonhuman primates. The investigators first created siRNAs that targeted *apoB* gene expression (apoB-siRNAs) on the basis of the known sequence of the gene. The apoB-siRNAs were synthesized in the laboratory and consisted of 21 nucleotides on the sense strand (the strand that was complementary to the apoB mRNA) and 23 nucleotides on the complementary antisense strand, with a two-nucleotide overhang.

The next task was to get the apoB siRNAs into the animals' cells. Although siRNAs are readily taken up by the cells of invertebrates such as *C. elegans*, most siRNAs will not readily pass through the

membranes of mammalian cells in a form that is still effective in gene silencing. In addition, siRNAs are rapidly removed from circulation. To overcome these problems, Zimmermann and her colleagues encapsulated the apoB siRNAs in lipids, creating stable nucleic-acid–lipid particles (SNALPs). The SNALPs greatly increased the time spent by the siRNAs in circulation and enhanced their uptake by the cells.

The researchers then tested the effects of the apoB-siRNAs on the synthesis of the ApoB protein and on cholesterol levels. They injected cynomolgus monkeys (**Figure 19.30a**) with SNALPs containing apoB siRNAs at two doses: 1 mg/kg and 2.5 mg/kg. In addition, they injected a third group of monkeys with saline as a control. The apoB siRNAs clearly silenced the *apoB* gene: 48 hours after treatment, apoB mRNA in the liver was reduced by 68% for monkeys receiving the 1-mg dose and 90% for monkeys receiving the 2.5-mg dose.



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Part a: Tony Camacho/Science Source.

19.30 Transfer of siRNAs for the ApoB protein into cynomolgus monkeys significantly lowered blood-cholesterol levels. (a) Cynomolgus monkey. (b) Different groups of monkeys were given saline (control), a 1-mg/kg dose, or a 2.5-mg/kg dose of siRNAs. [Part b: Data from T. S. Zimmerman, *Nature* 441:112, 2006, Figure 3b.]

Description

Part “a” shows a photo of a monkey sitting on the branch of a tree. Part “b” shows a bar graph that plots relative serum cholesterol in percent predose against time in hours. The horizontal axis shows time in hours as follows: 24, 48, 144, and 256. The vertical axis shows relative serum cholesterol (percent predose) ranging from 0 to 120, in increments of 20. It shows data for saline, 1 m g per k g, and 2 point 5 m g per k g. Approximate data are as follows:

24: saline, 85; 1 m g per k g, 78; and 2 point 5 m g, 62.

48: saline, 90; 1 m g per k g, 72; and 2 point 5 m g, 52.

144: saline, 108; 1 m g per k g, 85; and 2 point 5 m g, 42.

264: saline, 110; m g per k g, 90; and 2 point 5 m g, 39.

The bar denoting saline, 110, has a caption that reads, Serum cholesterol remained high in monkeys receiving saline. The bar denoting 2 point 5 m g, 39, has a caption that reads, serum cholesterol was greatly reduced in monkeys receiving the higher dose of s I R N A.

When the researchers examined serum cholesterol levels in the monkeys, they found that monkeys receiving the apoB siRNAs had significant reductions in blood-cholesterol levels (Figure 19.30b). Importantly, they observed no negative effects of the siRNA treatment. Although preliminary, this study suggests that siRNAs have the potential for future treatment of human diseases.

19.7 Biotechnology Harnesses the Power of Molecular Genetics

In addition to providing valuable new information about the nature and function of genes, molecular genetic techniques have many practical applications, including the production of pharmaceutical products and other chemicals, specialized bacteria, agriculturally important plants, and genetically engineered farm animals. These techniques are also used extensively in medical testing and are even being used to correct human genetic defects. Hundreds of companies now specialize in developing products through genetic engineering, and many large multinational corporations have invested enormous sums of money in molecular genetic research. As discussed earlier, DNA analysis is also used in criminal investigations and for the identification of human remains.

Pharmaceutical Products

The first commercial products to be developed with the use of genetic engineering were pharmaceutical products used in the treatment of human diseases and disorders. In 1979, Eli Lilly and Company began selling human insulin produced with the use of recombinant DNA technology. The gene for human insulin was inserted into plasmids and transferred to bacteria, which then

produced human insulin. Previously, diabetes was treated with insulin isolated from pig and cow pancreases; a few patients developed allergic reactions to this foreign protein. Insulin produced by recombinant bacteria has the advantage of being the same as that produced in the human body. Other pharmaceutical products produced through recombinant DNA technology include human growth hormone (for children with growth deficiencies) and tissue plasminogen activator (used to dissolve blood clots in heart-attack patients).

Specialized Bacteria

Bacteria play important roles in many industrial processes, including the production of ethanol from plant material, the leaching of minerals from ore, and the treatment of sewage and other wastes. The bacteria used in these processes have been modified by genetic engineering so that they work more efficiently. New strains of bacteria are being developed that will break down toxic chemicals and pollutants, enhance oil recovery, increase nitrogen uptake by plants, and inhibit the growth of pathogenic bacteria and fungi.

Agricultural Products

Recombinant DNA technology has had a major effect on agriculture, where it is now used to create crop plants and domestic animals with valuable traits. For example, plant pathologists had recognized for many years that plants infected with mild strains of viruses are resistant to infection by virulent strains. Using this knowledge, geneticists created plants with resistance to viruses by transferring genes for viral proteins to the plant cells. A genetically engineered squash, called Freedom II, carries genes from the watermelon mosaic virus 2 and the zucchini yellow mosaic virus, which protect the squash against viral infections. In 2017, the Arctic apple was introduced, a genetically modified apple that does not brown upon exposure to air.

Another objective has been to genetically engineer pest resistance into plants to reduce dependence on chemical pesticides. As discussed in Section 19.3, a gene from the bacterium *B. thuringiensis* that produces an insecticidal toxin has been transferred into corn, tomatoes, potatoes, cotton, and other plants. These Bt crops are now grown worldwide. Other genes that confer resistance to viruses and herbicides have been introduced into a number of crop plants; resistance to herbicides allows growers to use herbicides for weed control without harming the crops. During 2017, over 17 million farmers worldwide planted 190 million hectares of genetically engineered crops. In the United States, 92% of all corn, 94% of all cotton, and 94% of all soybeans grown in 2018 were genetically engineered.

Recombinant DNA technology is also being applied to domestic animals. The first animal to be genetically modified for food production in the United States—a rapidly growing salmon—was approved for commercial use in 2015. These salmon carry a foreign growth-hormone gene and promoter; the transgenic fish grow year-round instead of just during warm months, reaching market size more quickly and with less feed than wild salmon.

Transgenic animals are also being developed to carry genes that encode pharmaceutical products. Some eukaryotic proteins must be modified after translation, and only other eukaryotes (not bacteria) are capable of carrying out the modifications. For example, a gene for human clotting factor VIII was attached to the regulatory region of the sheep gene for β-lactoglobulin, a milk protein. The fused gene was injected into sheep embryos, creating transgenic sheep that produced the human clotting factor, which is used to treat hemophilia, in their milk. And through genetic engineering, scientists have created transgenic chickens that express a small RNA that blocks infection by avian influenza virus.

Highly efficient gene editing tools such as CRISPR-Cas systems and TALENs will increasingly be used to precisely edit the genomes of domestic plants and animals. For example, CRISPR-Cas9 has already been used to create transgenic wheat that is resistant to powdery mildew, a fungus that infects wheat. Because of its ease of use and low cost, CRISPR-Cas could be applied to less widely used

specialty crops and animals, potentially widening the use of genetically modified organisms. The ability to make very precise genetic changes with CRISPR-Cas, however, makes these alterations difficult to detect and track once they are made, raising questions about the labeling of products as genetically modified.

The genetic engineering of agricultural products is controversial. One area of concern focuses on the potential effects of releasing novel organisms produced by genetic engineering into the environment. There are many examples in which nonnative organisms released into a new environment have caused ecological disruption because they are free of their predators and other natural control mechanisms. Genetic engineering normally transfers only short sequences of DNA, which are small relative to the genetic differences that often exist between species, but even small genetic differences may alter ecologically important traits that might affect the ecosystem.

Another area of concern is the effect of genetically engineered crops on biodiversity. In the largest field test of genetically engineered plants ever conducted, scientists cultivated beets, corn, and rapeseed that were genetically engineered to resist herbicide along with traditional crops on 200 test plots throughout the United Kingdom. They then measured the biodiversity of native plants and animals in the plots. They found that the genetically engineered plants were highly successful in suppressing weeds; however, plots with genetically engineered beets and rapeseed had significantly

fewer insects that feed on weeds. For example, plots with genetically engineered rapeseed had 24% fewer butterflies than did plots with traditional crops.

There is also concern that transgenic organisms may hybridize with native organisms and transfer their genetically engineered traits. For example, herbicide resistance engineered into crop plants might be transferred to weeds, which would then be resistant to the herbicides that are now used for their control. Some studies have detected hybridization between genetically engineered crops and wild populations of plants. For example, evidence suggests that transgenic rapeseed (*Brassica napus*) has hybridized with the weed *Brassica rapa* in Canada. Other concerns focus on health-safety matters associated with the presence of engineered products in natural foods. Some critics have advocated required labeling of all genetically engineered foods that contain transgenic DNA or protein. Such labeling is required in countries of the European Union and will be required for some genetically modified foods in the United States starting in 2020.

On the other hand, the use of genetically engineered crops and domestic animals has potential benefits. It is predicted that the world's total crop production will need to increase by as much as 10% to meet the needs of an increased population, while at the same time climate change is predicted to lower crop yields in some areas. Genetically engineered plants with increased yields and the

ability to grow in currently unfavorable conditions may help to meet this future need. Genetically engineered crops that are pest resistant have the potential to reduce the use of environmentally harmful chemicals, and research findings indicate that lower amounts of pesticides are being used in the United States as a result of the adoption of transgenic plants. Studies conducted in China show that when Bt crops are used, farmers spray less chemical insecticide, allowing more predatory insects to survive and provide natural pest control. Transgenic crops also increase yields, providing more food per acre, which reduces the amount of land that must be used for agriculture. Genetically engineered plants offer the potential for the greater yields that may be necessary to feed the world's future population.

THINK-PAIR-SHARE Question 6

CONCEPTS

Recombinant DNA technology is used to create a wide range of commercial products, including pharmaceutical products, specialized bacteria, genetically engineered crops, and transgenic domestic animals.

✔ CONCEPT CHECK 12

What are some of the concerns about genetically engineered crops?

Genetic Testing

The identification and cloning of many important disease-causing human genes have allowed the development of probes for detecting disease-causing mutations. Prenatal testing is already available for many genetic disorders (see Section 6.4). Additionally, presymptomatic genetic tests for adults and children are available for an increasing number of disorders. Some genetic tests are now being offered directly to consumers, without requiring the participation of a health-care provider. These direct-to-consumer genetic tests are potentially available for many genetic conditions. The U.S. Food and Drug Administration has limited the sale of direct-to-consumer genetic tests for medical conditions, and today, only a few such medical tests are available in the United States.

The growing availability of genetic tests raises a number of ethical and social questions. For example, is it ethical to test for genetic diseases for which there is no cure or treatment? Other ethical and legal questions concern the confidentiality of test results. Who should have access to the results of genetic testing? Should relatives who also might be at risk be informed of the results of genetic testing?

Another set of concerns is related to the accuracy of genetic tests. For many genetic diseases, the only predictive tests available are those that identify a *predisposing* mutation in DNA, but many genetic

diseases can be caused by dozens or hundreds of different mutations. Probes that detect common mutations can be developed, but they will fail to detect rare mutations and may give a false negative result. Short of sequencing the entire gene—which is often too expensive—there is no way to identify all predisposed people. These questions and concerns are currently the focus of intense debate by ethicists, physicians, scientists, and patients.

Gene Therapy

Perhaps the ultimate application of recombinant DNA technology is **gene therapy**, the direct transfer of genes into human patients to treat disease. Today, thousands of patients have received gene therapy as a part of research trials. Gene therapy has been used as an experimental treatment for genetic diseases, cancer, heart disease, and even some infectious diseases, such as AIDS. Gene therapy products were approved for sale in the United States in 2017. A number of different methods for transferring genes into human cells are currently under development. Commonly used vectors include genetically modified retroviruses, adenoviruses, and adeno-associated viruses (Table 19.5). Researchers are exploring the use of CRISPR-Cas systems in gene therapy.

TABLE 19.5 Vectors used in gene therapy

Vector	Advantages	Disadvantages
Retrovirus	Efficient transfer	Transfers DNA only to dividing

		cells, inserts randomly; risk of producing wild-type viruses
Adenovirus	Transfers to nondividing cells	Causes immune reaction
Adeno-associated virus	Does not cause immune reaction	Holds small amount of DNA; hard to produce
Herpes virus	Can insert into cells of nervous system; does not cause immune reaction	Hard to produce in large quantities
Lentivirus	Can accommodate large genes	Safety concerns
Liposomes and other lipid-coated vectors	No replication; does not stimulate immune reaction	Low efficiency
Direct injection	No replication; directed toward specific tissues	Low efficiency; does not work well within some tissues
Pressure treatment	Safe, because tissues are treated outside the body and then transplanted into the patient	Most efficient with small DNA molecules
Gene gun (DNA coated on small gold particles and shot into tissue)	No vector required	Low efficiency

Information from E. Marshall, Gene therapy's growing pains, *Science* 269:1050–1055, 1995.

In spite of the growing number of clinical trials for gene therapy, significant problems remain in transferring foreign genes into human cells, getting them expressed, and limiting immune responses to the gene products and the vectors used to transfer the genes. There are also concerns about the safety of gene therapy. In 1999, a patient participating in a gene-therapy trial had a fatal immune reaction after he was injected with a viral vector carrying a gene to treat his metabolic disorder. In addition, five children who underwent gene therapy for severe combined immunodeficiency

disease developed leukemia that appeared to be directly related to the insertion of the retroviral vector into cancer-causing genes.

Despite these setbacks, gene-therapy research has moved ahead, and gene therapy has been used to treat a number of diseases, often with successful outcomes for the patients. Recent clinical trials include the use of gene therapy to treat hemophilia (see Chapter 6) by transferring a functional gene for clotting factor into patients using an adeno-associated virus. In another case, gene therapy was used to treat a child with epidermolysis bullosa, a devastating genetic skin disorder, by transferring a corrected copy of the *LAMB3* gene to the patient's skin cells, grown in culture, and then grafting the patches of genetically transformed skin onto the child. Gene therapy has been used to successfully treat children with spinal muscular atrophy (SMA), a fatal genetic disease involving nerve cells. And in 2017, for the first time researchers used gene editing technology to directly alter genes within a patient's body. They infused a patient with Hunter syndrome (a metabolic disease in which individuals lack an enzyme that breaks down polysaccharides) with viruses carrying a gene that encoded engineered zinc-finger nucleases and DNA consisting of a corrected copy of a gene.

Gene therapy has usually targeted only nonreproductive, or somatic, cells. Correcting genetic defects in these cells (termed *somatic gene therapy*) may provide positive benefits to patients but

will not affect the genes of future generations. Gene therapy that alters reproductive, or germ-line, cells (termed *germ-line gene therapy*) is technically possible but raises a number of significant ethical issues because it has the capacity to alter the gene pool of future generations.

THINK-PAIR-SHARE Question 7

CONCEPTS

Gene therapy is the direct transfer of genes into humans to treat disease. Gene therapy is now being used to treat genetic diseases, cancer, and infectious diseases.

✔ CONCEPT CHECK 13

What is the difference between somatic gene therapy and germ-line gene therapy?

Chapter 19 Review

CHAPTER SUMMARY

Section 19.1 Learning Objective: Be able to describe the challenges of working at the molecular level and some key innovations that have made this possible.

- Techniques of molecular genetics are used to locate, analyze, alter, sequence, study, and recombine DNA sequences. Four key innovations that have revolutionized genetics are recombinant DNA technology, the polymerase chain reaction, DNA sequencing technology, and genome editing methods.

Section 19.2 Learning Objective: Be able to describe molecular techniques that are used to cut and visualize DNA molecules.

- Recombinant DNA technology is a set of molecular techniques for locating, isolating, altering, and studying DNA segments.
- Restriction endonucleases are enzymes that make double-stranded cuts in DNA at specific base sequences.
- Engineered nucleases are proteins that are capable of making double-stranded cuts at specific DNA sequences. They include zinc-finger nucleases (ZFNs) and transcription activator–like effector nucleases (TALENs).

- The CRISPR-Cas9 system can be used to cut and edit the genome. It combines a single guide RNA with a nuclease, which together attach to DNA sequences and make double-stranded cuts at specific locations.
- CRISPR-Cas systems have been expanded and modified to carry out additional functions, such as cutting single-stranded DNA and RNA, making single base pair substitutions, stimulating and inhibiting gene expression, and detecting pathogens.
- DNA fragments can be separated by gel electrophoresis and visualized by staining the gel with a dye that is specific for nucleic acids or by labeling the fragments with a radioactive or chemical tag.

Section 19.3 Learning Objective: Be able to explain how DNA fragments can be copied with PCR and gene cloning.

- The polymerase chain reaction is a method for amplifying DNA enzymatically without cloning. A solution containing DNA is heated so that the two DNA strands separate; it is then quickly cooled, allowing primers to attach to the template DNA. The solution is then heated again, and DNA polymerase synthesizes new strands from the primers. Each time the cycle is repeated, the amount of DNA doubles.
- In gene cloning, a gene or a DNA fragment is placed into a bacterial cell, where it will be multiplied as the cell divides.
- Plasmids—small circular pieces of DNA—are often used as vectors to ensure that a cloned gene is stable and is replicated

within the recipient cells. Expression vectors contain sequences necessary for foreign DNA to be transcribed and translated.

Section 19.4 Learning Objective: Be able to describe how DNA sequences of interest can be located using gene libraries, in situ hybridization, and positional cloning.

- Genes can be isolated by creating a DNA library: a set of bacterial colonies or viral plaques that each contain a different cloned fragment of DNA. A genomic library contains the entire genome of an organism; a cDNA library contains DNA fragments complementary to the mRNAs expressed in a cell.
- In situ hybridization can be used to determine the chromosomal location of a gene or the distribution of the mRNA produced by a gene.
- Positional cloning uses linkage relations to determine the location of genes without any knowledge of their products.

Section 19.5 Learning Objective: Be able to describe how the base sequence of a DNA fragment is determined.

- The dideoxy (Sanger) method of DNA sequencing uses special substrates for DNA synthesis (dideoxyribonucleoside triphosphates, or ddNTPs) that terminate synthesis after they are incorporated into the newly synthesized DNA.
- Next-generation and third-generation sequencing methods sequence many DNA fragments simultaneously, providing a much faster and less expensive determination of a DNA sequence.

- Third-generation sequencing methods sequence single DNA molecules in real time.
- Short tandem repeats (STRs), or microsatellites, are used to identify people through DNA fingerprinting.

Section 19.6 Learning Objective: Be able to describe molecular techniques that are used for analyzing gene function, including targeted mutagenesis, transgenic animals, and RNAi.

- Forward genetics begins with a phenotype and conducts analyses to locate the responsible genes. Reverse genetics starts with a DNA sequence and conducts analyses to determine its phenotypic effect.
- Targeted mutagenesis can be used to produce mutations at specific sites in DNA, allowing genes to be tailored for a particular purpose.
- Transgenic animals, produced by injecting DNA into fertilized eggs, contain foreign DNA that is integrated into a chromosome. Knockout mice are mice in which a normal gene is disabled.
- RNA interference is used to silence the expression of specific genes.

Section 19.7 Learning Objective: Describe how techniques of molecular genetics have been used for practical applications.

- Techniques of molecular genetics are being used to create products of commercial importance, to develop diagnostic tests, and to treat diseases.

- In gene therapy, diseases are being treated by altering the genes of human cells.

WORKED PROBLEMS

Problem 1

A molecule of double-stranded DNA that is 5 million base pairs long has a base composition that is 62% G + C. How many times, on average, are restriction sites for the following restriction enzymes likely to be present in this DNA molecule?

a. *Bam*HI (recognition sequence is GGATCC)
b. *Hin*dIII (recognition sequence is AAGCTT)
c. *Hpa*II (recognition sequence is CCGG)

» Solution Strategy

What information is required in your answer to the problem?

The number of restriction sites likely to be present in the DNA molecule for each of the specified restriction enzymes.

What information is provided to solve the problem?

- The size of the DNA molecule.
- The G + C base composition of the DNA molecule.

- The recognition sequence for each restriction enzyme.

For help with this problem, review:

Restriction Enzymes in Section 19.2.

» Solution Steps

Hint: If you know the percentage of any base in the DNA, you can determine the percentages of all the other bases because G = C and A = T.

The percentages of G and C are equal in double-stranded DNA, so if G + C = 62%, then %G = %C = 62%/2 = 31%. The percentage of A + T = (100%−G−C)= 38%, and %A = %T = 38%/2 = 19%. To determine the probability of finding a particular base sequence, we use the multiplication rule (see Section 3.2), multiplying together the probability of finding each base at a particular site.

Recall: The multiplication rule states that the probability of two or more independent events is calculated by multiplying their independent probabilities.

a. The probability of finding the sequence GGATCC = 0.31 × 0.31 × 0.19 × 0.19 × 0.31 × 0.31 = 0.0003333. To determine the average number of GGATCC recognition sequences in a 5-million-base-pair piece of DNA, we multiply 5,000,000 bp × 0.00033 = 1666.5 recognition sequences.

b. The number of AAGCTT recognition sequences is 0.19 × 0.19 × 0.31 × 0.31 × 0.19 × 0.19 × 5,000,000 = 626 recognition sequences.
c. The number of CCGG recognition sequences is 0.31 × 0.31 × 0.31 × 0.31 × 5,000,000 = 46,176 recognition sequences.

Problem 2

You are given the following DNA fragment to sequence: 5′–GCTTAGCATC–3′. You first clone the fragment in bacterial cells to produce sufficient DNA for sequencing. You isolate the DNA from the bacterial cells and carry out the dideoxy-sequencing method. You then separate the products of the polymerization reactions by gel electrophoresis. Draw the bands that should appear on the gel from the four sequencing reactions.

» Solution Strategy

What information is required in your answer to the problem?

The positions of the bands on the sequencing gel.

What information is provided to solve the problem?

The base sequence of the DNA fragment to be sequenced.

For help with this problem, review:

Dideoxy Sequencing in Section 19.5.

» Solution Steps

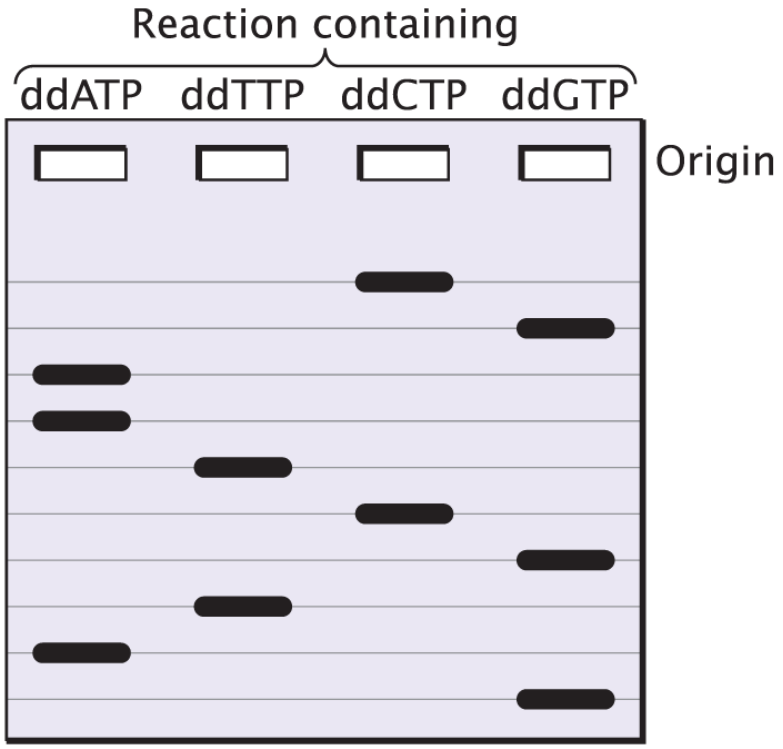
The first task is to write out the sequence of the newly synthesized fragment, which will be complementary and antiparallel to the original fragment. The original sequence is 5′–GCTTAGCATC–3′, so the newly synthesized sequence will be:

Original (template) sequence: 5′–GCTTAGCATC–3′
Newly synthesized sequence: 3′–CGAATCGTAG–5′

Recall: In dideoxy sequencing, a new DNA strand is synthesized, and that strand is what is sequenced. Therefore, the bands that appear on the gel represent the complement of the original sequence.

Thus, the sequence of the newly synthesized strand, written 5′→3′, is 5′–GATGCTAAGC–3′. Bands representing this sequence will appear on the gel, with the bands representing nucleotides near the 5′ end of the molecule at the bottom of the gel.

Hint: The smallest fragments, those nearest the 5′ end of the newly synthesized strand, will migrate most rapidly and will appear closest to the bottom (negative end) of the gel.



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

Description

Running horizontally through the four lanes are 10 lines, drawn as an aid to emphasize the order of bands in the gel. In the gel below each dideoxynucleotide is a rectangle, all of which are labeled Origin. Line 1 is the top of the gel. The d d A T P lane has 3 bands at lines 3, 4, and 9 positions. The d d T T P lane has 2 bands at lines 5 and 8 positions. The d d C T P lane has 2 bands at lines 1 and 6 positions and the d d G T P lane has 3 bands at lines 2, 7, and 10 positions.

IMPORTANT TERMS

biotechnology
recombinant DNA technology
genetic engineering

restriction enzyme
restriction endonuclease
cohesive end
engineered nuclease
zinc-finger nuclease (ZFN)
transcription activator–like effector nuclease (TALEN)
CRISPR-Cas system
gel electrophoresis
probe
Southern blotting
Northern blotting
Western blotting
gene cloning
polymerase chain reaction (PCR)
Taq polymerase
reverse-transcription PCR
environmental DNA (eDNA)
real-time PCR
cloning vector
linker
cosmid
bacterial artificial chromosome (BAC)
expression vector
yeast artificial chromosome (YAC)
Ti plasmid
DNA library
genomic library
cDNA library
in situ hybridization

positional cloning
chromosome walking
DNA sequencing
dideoxyribonucleoside triphosphate (ddNTP)
next-generation sequencing technology
DNA fingerprinting
microsatellite
short tandem repeat (STR)
forward genetics
reverse genetics
targeted mutagenesis
site-directed mutagenesis
oligonucleotide-directed mutagenesis
transgene
knockout mouse
knock-in mouse
gene therapy

ANSWERS TO CONCEPT CHECKS

1. Restriction enzymes exist naturally in bacteria, which use them in defense against viruses.
2. c
3. Southern blotting is used to transfer DNA from a gel to a solid medium. Northern blotting is used to transfer RNA from a gel to a solid medium, and Western blotting is used to transfer protein from a gel to a solid medium.

4. A heat-stable DNA polymerase is important to the success of PCR because the first step of the reaction requires that the solution be heated to 90°–100°C to separate the two DNA strands. Most enzymes are denatured at this temperature. With the use of a heat-stable polymerase, the enzyme can be added at the beginning of the reaction and will function throughout multiple cycles.
5. The gene and plasmid are cut with the same restriction enzyme and mixed together. DNA ligase is used to seal nicks in the sugar–phosphate backbone.
6. With the use of the genetic code and the amino acid sequence of the protein, possible nucleotide sequences that cover a small region of the gene can be deduced. A mixture of all the possible nucleotide sequences that might encode the protein, taking into consideration synonymous codons, is used to probe the library. To minimize the number of sequences required, a region of the protein that has relatively little degeneracy in its codons is selected.
7. The expression pattern of the genes can be examined, and the coding region of copies of the genes from individuals with the mutant phenotype can be compared with the coding region of wild-type individuals.
8. d
9. By using PCR with primers that flank the region containing tandem repeats.
10. b
11. c
12. Possible concerns include (a) ecological damage caused by introducing novel organisms into the environment, (b) negative

effects of transgenic organisms on biodiversity, (c) possible spread of transgenes to native organisms by hybridization, and (d) health effects of eating genetically modified foods.

13. Somatic gene therapy modifies genes only in somatic tissue, and these modifications cannot be inherited. Germ-line gene therapy alters genes in germ-line cells; these alterations have the potential to be passed on to future generations.

Chapter 19 Assessment

COMPREHENSION QUESTIONS

Section 19.1

1. List some of the effects and practical applications of molecular genetic analyses.

Section 19.2

2. What feature is commonly seen in the sequences recognized by restriction enzymes?

3. What normal role do restriction enzymes play in bacteria? How do bacteria protect their own DNA from the action of restriction enzymes?

4. How is CRISPR-Cas used to introduce specific changes into DNA sequences?

5. What are some ways that CRISPR-Cas systems have been enhanced and modified to provide additional functions?

6. What advantages do CRISPR-Cas systems have over restriction enzymes and engineered nucleases for editing DNA?

7. Explain how gel electrophoresis is used to separate DNA fragments of different lengths.

8. After DNA fragments have been separated by gel electrophoresis, how can they be visualized?

Section 19.3

9. Briefly explain how the polymerase chain reaction is used to amplify a specific DNA sequence. What are some of the limitations of PCR?

10. What is real-time PCR?

11. Give three important characteristics of cloning vectors.

12. Briefly describe the methods for inserting foreign DNA into plasmids that were described in this chapter.

13. Briefly explain how an antibiotic-resistance gene and the *lacZ* gene can be used to determine which cells contain a particular plasmid.

Section 19.4

14. How does a genomic library differ from a cDNA library?

15. How are probes used to screen DNA libraries? Explain how a synthetic probe can be prepared when the protein product of a gene is known.

16. Briefly explain in situ hybridization, giving some applications of this technique.

17. Briefly explain how a gene can be isolated through positional cloning.

18. Explain how chromosome walking can be used to find a gene.

Section 19.5

19. What is the purpose of the dideoxyribonucleoside triphosphates in the dideoxy-sequencing reaction?

20. What advantages do next-generation sequencing technologies have over dideoxy sequencing?

21. What are advantages and disadvantages of third-generation sequencing over next-generation sequencing?

22. What is DNA fingerprinting? What types of sequences are examined in DNA fingerprinting?

Section 19.6

23. How does a reverse genetic approach differ from a forward genetic approach?

24. Briefly explain how site-directed mutagenesis is carried out.

25. What are knockout mice, how are they produced, and for what are they used?

26. How is RNA interference used in the analysis of gene function?

Section 19.7

27. What is gene therapy?

APPLICATION QUESTIONS AND PROBLEMS

Section 19.2

28. CRISPR-Cas9 was first developed as a molecular tool in 2012. During the next few years, its use in molecular biology exploded; scientists around the world began applying it to many different research problems, and hundreds of research papers describing its application were published. Explain why CRISPR-Cas is such a powerful tool in molecular genetics.

29. *Suppose that a geneticist discovers a new restriction enzyme in the bacterium *Aeromonas ranidae*. This restriction enzyme is the first to be isolated from this bacterial species. Using the standard convention for abbreviating restriction enzymes, give this new restriction enzyme a name (for help, see footnote to **Table 19.1**).

30. How often, on average, would you expect a restriction endonuclease to cut a DNA molecule if the recognition sequence for the enzyme had 5 bp? (Assume that the four types of bases are equally likely to be found in the DNA and that the bases in a recognition sequence are independent.) How often would the endonuclease cut the DNA if the recognition sequence had 8 bp?

31. *A microbiologist discovers a new restriction endonuclease. When DNA is digested by this enzyme, fragments that average 1,048,500

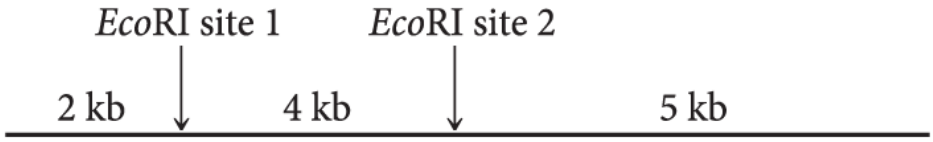
bp in length are produced. What is the most likely number of base pairs in the recognition sequence of this enzyme?

32. Will restriction sites for an enzyme that has 4 bp in its recognition sequence be closer together, farther apart, or similarly spaced, on average, compared with those of an enzyme that has 6 bp in its recognition sequence? Explain your reasoning.

33. CONNECT What aspect of DNA structure (see Chapter 10) causes DNA to migrate within an electrophoretic gel?

34. *About 60% of the base pairs in the human genome are AT. If the human genome has 3.2 billion base pairs of DNA, about how many times will the following restriction sites be present?
    a. *Bam*HI (recognition sequence is 5′–GGATCC–3′)
    b. *Eco*RI (recognition sequence is 5′–GAATTC–3′)
    c. *Hae*III (recognition sequence is 5′–GGCC–3′)

35. *A linear piece of DNA has the following *Eco*RI restriction sites:



Description
Going left to right, the D N A is labeled 2 k b, restriction site 1, 4 k b, restriction site 2, and 5 k b.

    a. This piece of DNA is cut by *Eco*RI, the resulting fragments are separated by gel electrophoresis, and the gel is stained with

ethidium bromide. Draw a picture of the bands that will appear on the gel.

b. If a mutation that alters *Eco*RI site 1 occurs in this piece of DNA, how will the banding pattern on the gel differ from the one that you drew in part *a*?
c. If mutations that alter *Eco*RI sites 1 and 2 occur in this piece of DNA, how will the banding pattern on the gel differ from the one that you drew in part *a*?
d. If 1000 bp of DNA were inserted between the two restriction sites, how would the banding pattern on the gel differ from the one that you drew in part *a*?
e. If 500 bp of DNA between the two restriction sites were deleted, how would the banding pattern on the gel differ from the one that you drew in part *a*?

36. CONNECT What are some ways that bacterial defense systems (see Section 9.4 in Chapter 9) have been adapted for use as tools in molecular genetic analysis? Why do bacterial defense systems make good tools for manipulating DNA?

Section 19.3

37. Compare and contrast the use of PCR and gene cloning for amplifying DNA fragments. What are the advantages and disadvantages of each method?

38. CONNECT How is the DNA synthesis that occurs in PCR similar and different from the DNA synthesis that occurs in replication?

(See Chapter 12 for details of DNA synthesis in replication.)

39. *Which vectors (plasmid, phage λ, cosmid, bacterial artificial chromosome) can be used to clone a continuous fragment of DNA with the following lengths?
    a. 4 kb
    b. 20 kb
    c. 35 kb
    d. 100 kb

40. A geneticist uses a plasmid for cloning that has the *lacZ* gene and a gene that confers resistance to penicillin. The geneticist inserts a piece of foreign DNA into a restriction site that is located within the *lacZ* gene and uses the plasmid to transform bacteria. Explain how the geneticist can identify bacteria that contain a copy of a plasmid with the foreign DNA.

41. CONNECT Bacterial plasmids (see Chapter 9) have been genetically engineered to serve as cloning vectors for bacteria. What characteristics of plasmids make them useful as cloning vectors?

Section 19.4

42. Suppose that you have just graduated from college and have started working at a biotechnology firm. Your first job assignment is to clone the pig gene for the hormone prolactin. Assume that the pig gene for prolactin has not yet been isolated, sequenced, or mapped; however, the mouse gene for prolactin has been cloned,

and the amino acid sequence of mouse prolactin is known. Briefly explain two different strategies that you might use to find and clone the pig gene for prolactin.

43.

Fabian von Poser/imageBROKER/
Age Fotostock.

CONNECT *A molecular biologist wants to isolate a gene from a scorpion that encodes the deadly toxin found in its stinger, with the ultimate purpose of transferring this gene to bacteria and producing the toxin for use as a commercial pesticide. Isolating the gene requires a DNA library. Should the molecular biologist create a genomic library or a cDNA library? Explain your reasoning.

44. *A protein has the following amino acid sequence:

Met-Tyr-Asn-Val-Arg-Val-Tyr-Lys-Ala-Lys-Trp-Leu-Ile-His-Thr-Pro

You wish to make a set of probes to screen a cDNA library for the sequence that encodes this protein. Your probes should be at least 18 nucleotides in length.

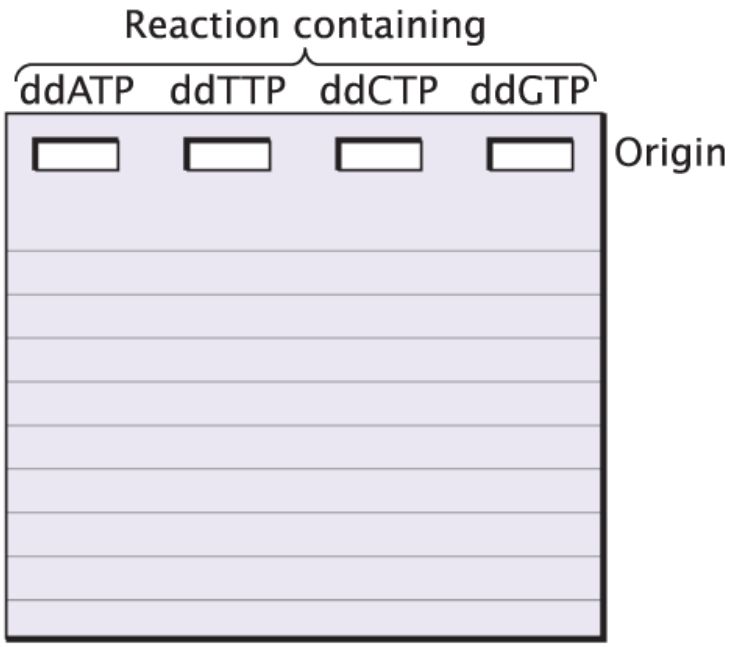
a. Which amino acids in the protein should be used to construct the probes so that the least degeneracy results? (Consult the genetic code in **Figure 15.10**.)
b. How many different probes must be synthesized to be certain that you will find the cDNA sequence that specifies the protein?

Section 19.5

45. Suppose that you want to sequence the following DNA fragment:

$$5'\text{–TCCCGGGAAA-primer site–}3'$$

You first use PCR to amplify the fragment so that there is sufficient DNA for sequencing. You carry out dideoxy sequencing and then separate the products of the sequencing reactions by gel electrophoresis. Draw the bands that should appear on the gel from the four sequencing reactions.



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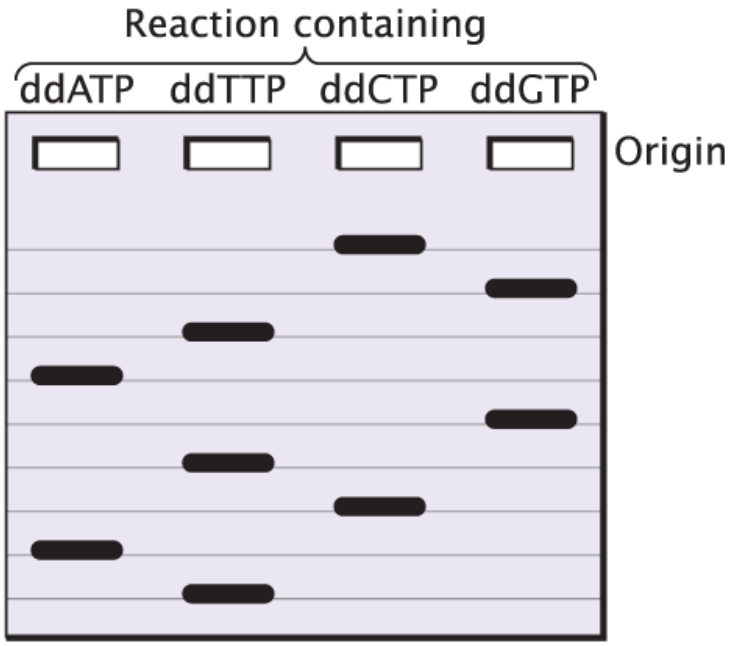
Description
The four lanes are labeled for each of the 4 dideoxynucleotides d d A T P, d d T T P, d d C T P, and d d G T P. In the gel below each is a rectangle, all of which are labeled Origin. The gel has no bands in it.

46. *Suppose that you are given a short fragment of DNA to sequence. You amplify the fragment with PCR and set up a series of four dideoxy reactions. You then separate the products of the reactions by gel electrophoresis and obtain the following banding pattern:



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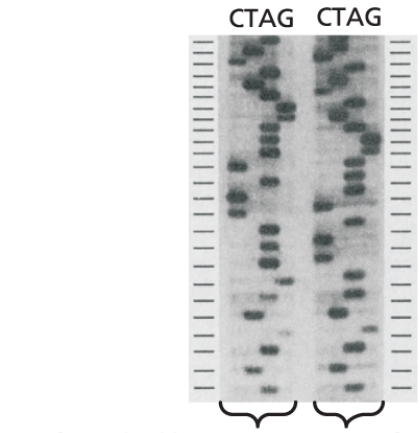
Description
Running horizontal through the gel are 9 lines, drawn as an aid to emphasize the order of bands in the gel. In the gel below each dideoxynucleotide is a rectangle, all of which are labeled Origin. Line 1 is the top of the gel. Lane d d A T P has bands at lines 4 and 8 positions. Lane d d T T P has bands at lines 3, 6, and 9 positions. Lane d d C T P has bands at lines 1 and 7 positions. And lane d d G T P has bands at lines 2 and 5 positions.

Write out the base sequence of the original fragment that you were given.

Original sequence: 5′–_____–3′

47. DATA ANALYSIS The accompanying photo shows a sequencing gel from the original study that first sequenced the cystic fibrosis gene (J. R. Riordan et al. 1989. *Science* 245:1066–1073). From the photo, determine the sequence of the normal copy of the gene and the

sequence of the mutated copy of the gene. Identify the location of the mutation that causes cystic fibrosis. (Hint: The CF mutation is a 3-bp deletion.)



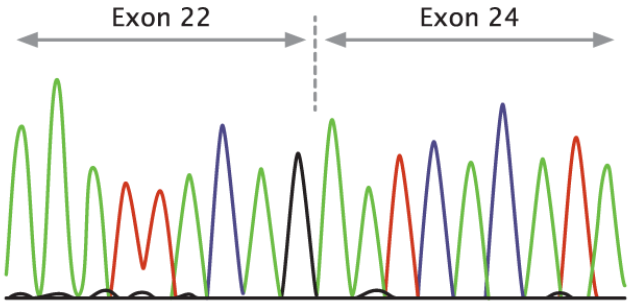
J. Riordan et al. 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA, *Science* 245:1066–1073, © 1989. Reprinted with permission from AAAS, permission conveyed through Copyright Clearance Center, Inc.

[J. Riordan et al. 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA, Science 245:1066–1073, © 1989. Reprinted with permission from AAAS, permission conveyed through Copyright Clearance Center, Inc.]

Description
Four bases are shown on top of the each gel column as follows: C, T, A, G. The healthy person has C's at lines 11, 12, 14, and 23, G's at lines 4, 7, 18, and 19, and T's at lines 2, 5, 21, and 24. The rest of the 13 bands are the A nucleotide. For the person with cystic fibrosis: Cs are at lines 8, 9, 11, 20 and 23, G's are at lines 4, 15, and 16, and T's at lines 2, 5, 18, 21, 24, and 25. The remaining 11 nucleotides are A's.

48. DATA ANALYSIS Shown below is the DNA sequencing chromatogram that researchers used to show that CRISPR-Cas genome editing had precisely removed exon 23 in *mdx* mice and eliminated a stop

codon responsible for muscular dystrophy in these mice (Long et al. *Science* 351:400–402; see introduction to this chapter). Write out the bases found in the DNA that was sequenced. (Hint: See **Figure 19.18** for determining which colors represent which bases.)



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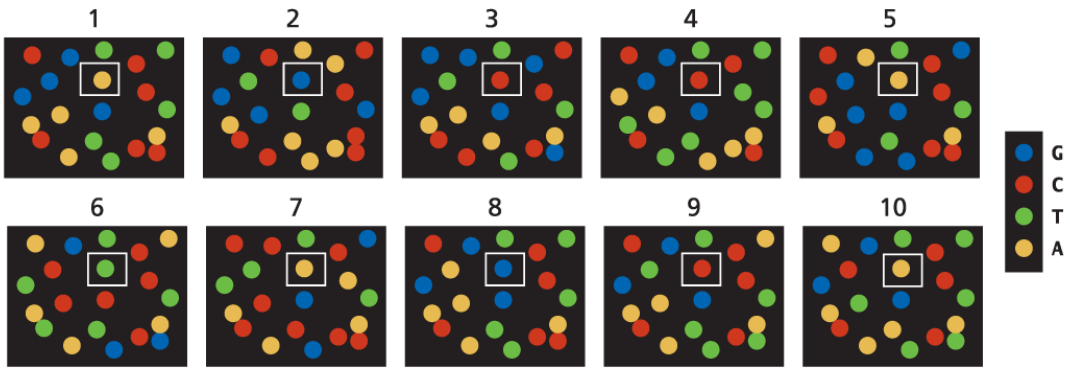
Description
First half of the chromatogram shows exon 22, while the other half shows exon 24. Several narrow concave down curves are shown for both the exons in the following colors: green, red, violet, and black. The peaks for exon 22 are: green, green, green, red, red, green, blue, green black; and for exon 24: green green red blue green blue green red green.A few curves in green have the highest peak for exon 22, while a few curves in violet have the highest peak for exon 24.

49. The following represents a small portion of the flow cell for Illumina sequencing. Ten cycles of sequencing are shown. If blue represents guanine, red represents cytosine, green represents thymine, and yellow represents adenine, write out the base sequence of the DNA fragment represented by the cluster of fragments enclosed in the box. Indicate the 5′ and 3′ ends of the fragment whose base sequence is being determined.



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Description
Ten black boxes are shown labeled “1” through “10”. A key shows circle colors for four nucleotide bases as follows: blue circle, G; red circle, C; green circle, T; and yellow circle, A. All the boxes have randomly distributed circles of these colors. In each box, one circle is enclosed within a square as follows going from 1 to 10: yellow, blue, red, red, yellow, green, yellow, blue, red, and yellow.

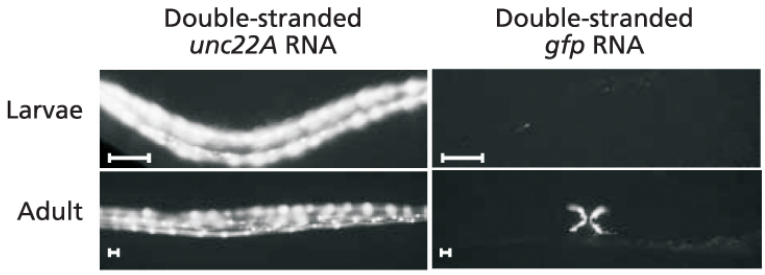
Section 19.6

50. CONNECT In his experiments on pea plants (see Chapter 3), did Mendel use a forward genetics approach or a reverse genetics approach? Explain your reasoning.

51. You have discovered a gene in mice that is similar to a gene in yeast. How might you determine whether this gene is essential for development in mice?

52. DATA ANALYSIS * Andrew Fire, Craig Mello, and their colleagues were among the first to examine the effects of double-stranded RNA (dsRNA) on gene expression (A. Fire et al. 1998. *Nature* 391:806–811). In one experiment, they used a transgenic strain of *C. elegans* into which a

gene (*gfp*) for a green fluorescent protein had been introduced. They injected some worms with double-stranded RNA complementary to coding sequences of the *gfp* gene and injected other worms with double-stranded RNA complementary to the coding region of a different gene (*unc22A*) that encodes a muscle protein. The accompanying photographs show larval and adult progeny of the injected worms. Green fluorescent protein appears as bright spots in the photographs.



Andrew Fire et al. 1998. Potent and specific genetic interference by doublestranded RNA in *Caenorhabditis elegans, Nature* 391:806–811. Reprinted by permission of Macmillan Publishers Ltd., © 1998, permission conveyed through Copyright Clearance Center, Inc.

[Andrew Fire et al. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans, Nature* 391:806–811. Reprinted by permission of Macmillan Publishers Ltd., © 1998, permission conveyed through Copyright Clearance Center, Inc.]

Description

There are four photos lined up 2 by 2. The top left photo is labeled larvae and double-stranded u n c 22 A R N A. This worm is completely lit up. The top right photo is labeled larvae and double stranded g f p R N A. Nothing in this worm is lit up. The bottom left box is labeled adult and double-stranded u n c 22 A R N A and the entire worm in lit up, but the signal is less intense than the first showing the larvae. The bottom right box is labeled adult and double-stranded g f p R N A. This box shows only a small part of the worm is lit up in the shape of an X.

a. Explain these results.
b. Fire and Mello conducted another experiment in which they injected double-stranded RNA complementary to the introns and promoter sequences of the *gfp* gene. What results would you expect with this experiment? Explain your answer.

CHALLENGE QUESTION

Section 19.6

53. Suppose that you are hired by a biotechnology firm to produce a strain of giant fruit flies by using recombinant DNA technology so that genetics students will not be forced to strain their eyes when looking at tiny flies. You go to the library and learn that growth in fruit flies is normally inhibited by a hormone called shorty substance P (SSP). You decide that you can produce giant fruit flies if you can somehow turn off the production of SSP. Shorty substance P is synthesized from a compound called XSP in a single-step reaction catalyzed by the enzyme runtase:

$$\text{XSP} \xrightarrow[\text{Runtase}]{} \text{SSP}$$

A researcher has already isolated cDNA for runtase and has sequenced it, but the location of the runtase gene in the *Drosophila* genome is unknown. In attempting to devise a strategy for turning off the production of SSP using standard recombinant DNA techniques, you discover that deleting, inactivating, or otherwise mutating this DNA sequence in *Drosophila* turns out to be

extremely difficult. Therefore, you must restrict your genetic engineering to gene augmentation (adding new genes to cells). Describe the methods that you will use to turn off SSP and produce giant flies.

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 19.1

1. What were some key innovations prior to Watson, Crick, Franklin, and Wilkins's discovery of the three-dimensional structure of DNA that were important in shaping the study of genetics?

Section 19.2

2. Now that you understand how the CRISPR-Cas9 system works, think back to the experiments discussed in the introduction to this chapter, in which researchers used CRISPR-Cas9 genome editing to treat mice with Duchenne muscular dystrophy. Why did the researchers choose to cut out the entire exon 23 in the mice with the disorder? Why not replace the specific mutation using a donor piece of DNA and homologous recombination? Propose some possible explanations.

3. Propose some additional modifications of CRISPR-Cas systems that might allow them to be used as RNA-guided devices for genetic analysis and function.

4. Some people have argued that editing the genomes of human embryos is ethically defensible as long as the embryos are not allowed to develop past an early stage and thus will never result in the birth of genetically modified humans. Others have argued that no genome editing should be carried out on human embryos. Present arguments for and against using genome editing on human embryos.

Section 19.3

5. Make a table comparing the advantages and disadvantages of PCR and gene cloning for amplifying DNA fragments.

Section 19.7

6. Much of the controversy over genetically engineered foods has centered on whether special labeling should be required on all products made from genetically modified crops. Some people have advocated labeling that identifies the product as having been made from genetically modified plants. Others have argued that food labeling should be required to identify only the ingredients, not the process by which they were produced. Choose a side in this issue, and justify your stand.

7. Why is it that somatic gene therapy is allowed in many countries, yet germ-line gene therapy is often restricted? What are some arguments for and against germ-line gene therapy?

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Draw a concept map that summarizes how researchers are using CRISPR-Cas to potentially treat boys with Duchenne muscular dystrophy. Include at least the following terms in your map: Duchenne muscular dystrophy, dystrophin gene, X-linked inheritance, gene therapy, stop codon, CRISPR-Cas, *mdx* mice, exon 23, exon skipping.

Section 19.2

2. Create a concept map that compares and contrasts restriction enzymes, engineered nucleases, and CRISPR-Cas.

Section 19.3

3. Compare the use of different cloning vectors by drawing a concept map that includes the following terms: cloning vector, plasmid vector, origin of replication, selectable markers, restriction site, cosmid, bacterial artificial chromosome, bacteriophage λ, expression vector, Ti plasmid.

Section 19.5

4. Draw a concept map that summarizes the different types of DNA sequencing. Include at least the following terms: DNA sequencing

methods, dideoxy sequencing, Illumina sequencing, PacBio sequencing, nanopore sequencing, third-generation sequencing, next-generation sequencing.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 20

Genomics and Proteomics

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The Earth BioGenome Project proposes to sequence the genomes of all of the Earth's eukaryotic species by 2028.

Sequencing Earth's Biodiversity

One of the great mysteries of life is how many species inhabit the Earth. Scientists estimate that there are at least 10–15 million eukaryotic species and perhaps trillions of bacteria and archaea,

but only about 2.3 million species have actually been described. Of these, fewer than 15,000—mostly bacteria, archaea, and fungi—have had their genomes completely or partially sequenced. Those sequenced genomes have provided tremendously valuable information that has advanced medicine, agriculture, biotechnology, and the study of evolution. In spite of these accomplishments, much of the world's biodiversity remains unknown.

Many of the Earth's unknown organisms may soon disappear. Today, much of the world's biodiversity is threatened by habitat loss, pollution, climate change, exploitation, and other human activities. Scientists estimate that many of the world's species are in decline and perhaps a third are approaching extinction. By 2050, up to 50% of the world's species may become extinct. Humans depend on wild species as sources of food, medicines, and materials, as well as to generate ecosystem services that give rise to clean water and air. The consequences of losing large amounts of our biodiversity are unknown but potentially catastrophic.

These threats have intensified efforts to find, identify, and study the Earth's biodiversity. Toward this end, scientists from around the world launched the Earth BioGenome Project (EBP) in 2018, with the audacious goal of sequencing all eukaryotic species found on Earth within the next 10 years. The overall aim of EBP is to "revise and reinvigorate our understanding of biology and evolution." Within this broad framework are several more specific goals,

including better understanding of the evolutionary relationships of all known organisms, discovering new species, clarifying how human activities affect species, and discovering new medicines and other useful products.

Because a parallel project—the Earth Microbiome Project—was already underway to sequence the world's microbial diversity, the leaders of EBP decided to focus on eukaryotic species. In 2017, there were only about 2500 eukaryotic species whose genomes had been completely or partially sequenced. Several research organizations have begun to sequence groups of eukaryotic organisms (Table 20.1). For example, the G10K project seeks to sequence all vertebrate genomes, and the 10KP project is sequencing 10,000 plant genomes. The leaders of EBP hope to work with and expand the efforts of these different groups, creating a coordinated global program to sequence and understand all forms of eukaryotic life.

TABLE 20.1 Projects to sequence the diversity of life

Project Name	Goal
G10K	Sequence all vertebrate genomes
GIGA	Sequence 7000 marine invertebrates
GAGA	Sequence genomes of 300 ant genera

i5K	Sequence 5000 arthropods
10KP	Sequence 10,000 plant genomes
Darwin Tree of Life Project	Sequence all 66,000 eukaryotic species found across the British Isles

Data from Table 2 in Lewin et al., *PNAS* 15:4325–4333, 2017.

The EBP's plan is to sequence approximately 1.5 million known eukaryotic species and collect environmental DNA samples to discover unknown species. In the first phase of the project, researchers will carry out sequencing of at least one representative species from each of the 9000 eukaryotic families, creating high-quality reference genomes for these organisms. Next, they will conduct lower-resolution sequencing of at least one species from each of the 150,000 known genera. In the final phase of the project, researchers will carry out low-resolution sequencing of all remaining previously known and newly discovered species, the genome sequences of which can then be improved by comparing them to the high-quality reference genomes created in phase I. The projected cost of the project is approximately $4.7 billion, which is actually less in today's dollars than the cost of the original sequencing of the human genome. Carrying out the goals of EBP will require coordination among thousands of scientists worldwide. The organizers envision that numerous citizen-scientists will also participate by collecting and cataloging of specimens to be sequenced.

In recent years, the cost of sequencing has dropped dramatically, so that an entire species genome can be sequenced for less than $1000. Leaders of the EBP believe that sequencing costs will continue to drop, making the project economically possible. One of the biggest hurdles, and perhaps the biggest expense, will be the collection of samples of all known species. Another challenge will be sequencing an estimated 140,000 species of protists, algae, and other single-celled eukaryotes, but recent advances in single-cell sequencing suggest that this is feasible. Regardless of whether the EBP reaches its goal of sequencing all eukaryotic species by 2028, thousands of eukaryotic genomes will no doubt be sequenced, greatly enriching our understanding of the abundant and beautiful life that surrounds us.

THINK-PAIR-SHARE

Although the organizers of the Earth BioGenome Project have suggested a number of potential benefits of sequencing all Earth's organisms, not everyone has been enthusiastic. Some people have questioned the high cost of the project and the benefit of sequencing all species versus a smaller group of selected species. Discuss the potential benefits of the Earth BioGenome Project, as well as these and other possible criticisms. Do you think the project is justified? Why or why not?

Genomics is the field of genetics that attempts to understand the content, organization, function, and evolution of the genetic information contained in whole genomes. The field of genomics is at the cutting edge of modern biology; information resulting from research in this field has made significant contributions to human

health, agriculture, and numerous other areas. It has provided gene sequences necessary for producing medically important proteins through recombinant DNA technology. Comparisons of genome sequences from different organisms are also leading to a better understanding of evolution and the history of life. Genomics provides the potential for manipulating genomes, or even creating genomes from scratch.

We begin this chapter by examining genetic and physical maps and methods for sequencing entire genomes. Next, we explore functional genomics—how genes are identified in genome sequences and how their functions are defined. The sequence of a genome by itself is of limited use, and now that the sequencing of genomes has become routine, much of genomics is focused on deciphering the functions of the sequences that are obtained. We also discuss how genome sequences and organization vary among organisms and how genomes evolve. In the final section of the chapter, we consider proteomics, the study of the complete set of proteins found in a cell.

20.1 Structural Genomics Determines the DNA Sequences and Organization of Entire Genomes

Structural genomics is the study of the organization and sequence of the genetic information contained within a genome, providing the basic DNA sequence information that is used in functional and evolutionary studies. An early step in characterizing a genome may be to prepare genetic and physical maps of its chromosomes. These maps provide information about the relative locations of genes, molecular markers, and chromosome segments, which can help in positioning chromosome segments and aligning stretches of sequenced DNA into a whole-genome sequence. These maps are also the foundation for positional cloning (see Section 19.4), in which genes that influence specific traits are located and sequenced.

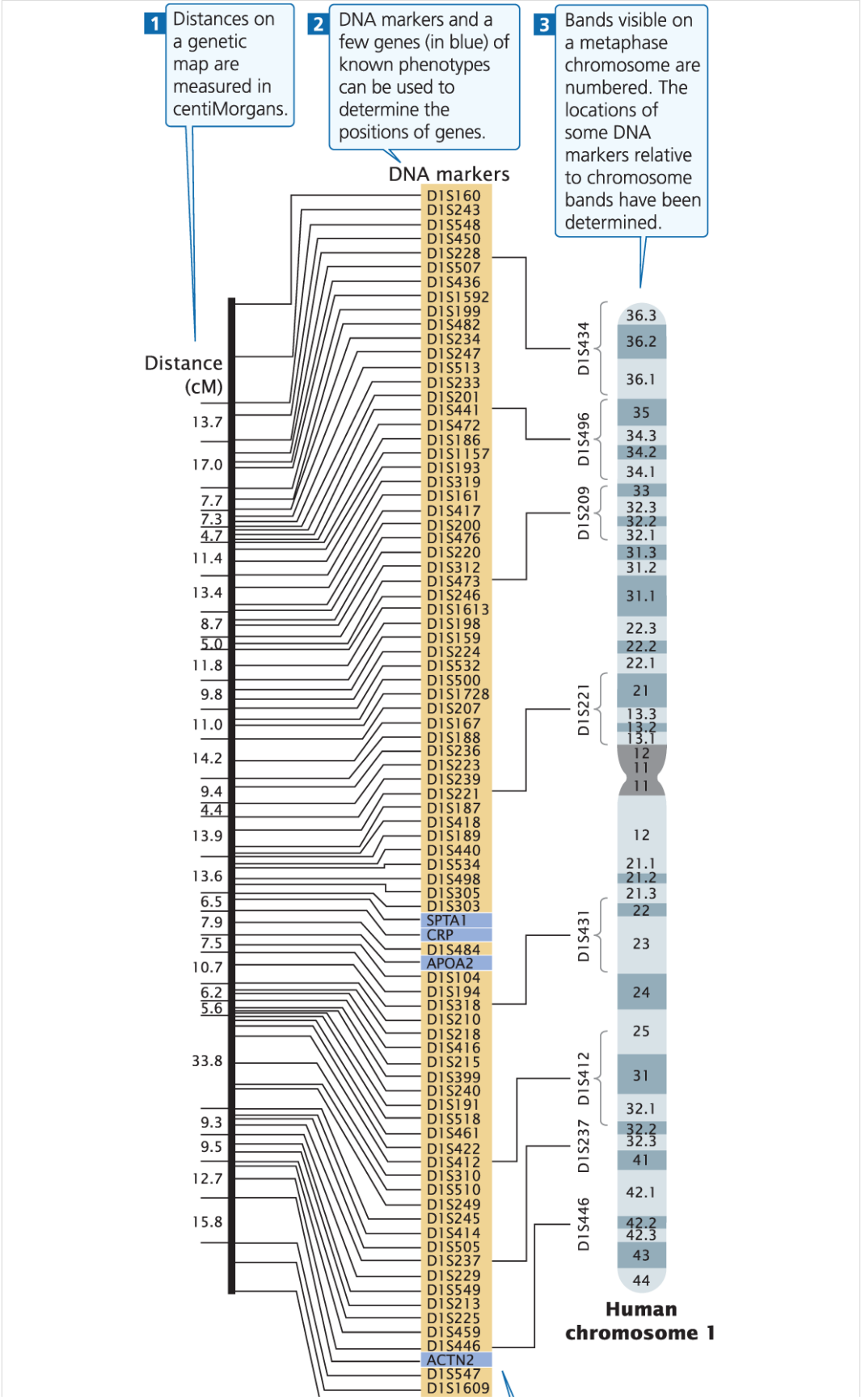
Genetic Maps

Everyone has used a map at one time or another. Maps are indispensable for finding a new friend's house, the way to an unfamiliar city in your state, or the location of a country. Each of these examples requires a map with a different scale. To find a friend's house, you would probably use a city street map or zoom in to a fine-scale city map that shows city streets; to find your way to an

unknown city, you might pick up a state highway map or zoom out to a larger view that shows cities within a state; to find a country such as Kazakhstan, you would need a world atlas or an even larger-scale map that shows different countries. Similarly, navigating a genome requires maps of different types and scales.

CONSTRUCTION OF GENETIC MAPS

Genetic maps (also called *linkage maps*) provide a rough approximation of the locations of genes relative to the locations of other known genes (**Figure 20.1**). These maps are based on the genetic process of recombination (hence the name *genetic map*). The basic principles of constructing genetic maps are discussed in detail in Chapter 7. In short, individual organisms of known genotype are crossed, and the frequency of recombination between loci is determined by examining the progeny. If the recombination frequency between two loci is 50%, then the loci are found on different chromosomes or are far apart on the same chromosome. If the recombination frequency is less than 50%, the loci are found close together on the same chromosome (they belong to the same linkage group). For linked genes, the rate of recombination is proportional to the physical distance between the loci. Distances on genetic maps are measured in recombination frequencies (centiMorgans [cM] or map units [m.u.]). Data from multiple two-point or three-point crosses can be integrated into linkage maps of whole chromosomes.


1 Distances on a genetic map are measured in centiMorgans.
2 DNA markers and a few genes (in blue) of known phenotypes can be used to determine the positions of genes.
3 Bands visible on a metaphase chromosome are numbered. The locations of some DNA markers relative to chromosome bands have been determined.
DNA markers
Distance (cM)
13.7
17.0
7.7
7.3
4.7
11.4
13.4
8.7
5.0
11.8
9.8
11.0
14.2
9.4
4.4
13.9
13.6
6.5
7.9
7.5
10.7
6.2
5.6
33.8
9.3
9.5
12.7
15.8
D1S160
D1S243
D1S548
D1S450
D1S228
D1S507
D1S436
D1S1592
D1S199
D1S482
D1S234
D1S247
D1S513
D1S233
D1S201
D1S441
D1S472
D1S186
D1S1157
D1S193
D1S319
D1S161
D1S417
D1S200
D1S476
D1S220
D1S312
D1S473
D1S246
D1S1613
D1S198
D1S159
D1S224
D1S532
D1S500
D1S1728
D1S207
D1S167
D1S188
D1S236
D1S223
D1S239
D1S221
D1S187
D1S418
D1S189
D1S440
D1S534
D1S498
D1S305
D1S303
SPTA1
CRP
D1S484
APOA2
D1S104
D1S194
D1S318
D1S210
D1S218
D1S416
D1S215
D1S399
D1S240
D1S191
D1S518
D1S461
D1S422
D1S412
D1S310
D1S510
D1S249
D1S245
D1S414
D1S505
D1S237
D1S229
D1S549
D1S213
D1S225
D1S459
D1S446
ACTN2
D1S547
D1S1609
D1S434
D1S496
D1S209
D1S221
D1S431
D1S412
D1S237
D1S446
36.3
36.2
36.1
35
34.3
34.2
34.1
33
32.3
32.2
32.1
31.3
31.2
31.1
22.3
22.2
22.1
21
13.3
13.2
13.1
12
11
11
12
21.1
21.2
21.3
22
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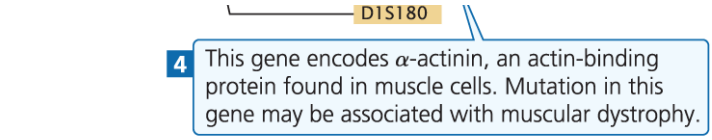
31
32.1
32.2
32.3
41
42.1
42.2
42.3
43
44
Human chromosome 1



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20.1 Genetic maps are based on rates of recombination. Shown here is a genetic map of human chromosome 1.

Description

The map on the left is a thick black vertical line with lines jutting out indicating the locations of genes and markers. CentiMorgan distances are labeled on the line as follows: 13 point 7, 17 point 0, 7 point 7, 7 point 3, 4 point 7, 11 point 4, 13 point 4, 8 point 7, 5 point 0, 11 point 8, 9 point 8, 11 point 0, 14 point 2, 9 point 4, 4 point 4, 13 point 9, 13 point 6, 6 point 5, 7 point 9, 7 point 5, 10 point 7, 6 point 2, 5 point 6, 33 point 8, 9 point 3, 9 point 5, 12 point 7, and 15 point 8. A caption reads, “Distances on a genetic map are measured in centiMorgans.” Center of the map shows various D N A markers as follows: D 1 S 160, D 1 S 243, D 1 S 548, D 1 S 450, D 1 S 228, D 1 S 507, D 1 S 436, D 1 S 1592, D 1 S 199, D 1 S 482, D 1 S 234, D 1 S 247, D 1 S 513, D 1 S 233, D 1 S 201, D 1 S 441, D 1 S 472, D 1 S 186, D 1 S 1157, D 1 S 193, D 1 S 319, D 1 S 161, D 1 S 417, D 1 S 200, D 1 S 476, D 1 S 220, D 1 S 312, D 1 S 473, D 1 S 246, D 1 S 1613, D 1 S 198, D 1 S 159, D 1 S 224, D 1 S 532, D 1 S 500, D 1 S 1728, D 1 S 207, D 1 S 167, D 1 S 188, D 1 S 236, D 1 S 223, D 1 S 239, D 1 S 221, D 1 S 187, D 1 S 418, D 1 S 189, D 1 S 440, D 1 S 534, D 1 S 498, D 1 S 305, D 1 S 303, S P T A 1, CRP, D 1 S 484, APOA2, D 1 S 104, D 1 S 194, D 1 S 318, D 1 S 210, D 1 S 218, D 1 S 416, D 1 S 215, D 1 S 399, D 1 S 240, D 1 S 191, D 1 S 518, D 1 S 461, D 1 S 422, D 1 S 412, D 1 S 310, D 1 S 510, D 1 S 249, D 1 S 245, D 1 S 414, D 1 S 505, D 1 S 237, D 1 S 229, D 1 S 549, D 1 S 213, D 1 S 225, D 1 S 459, D 1 S 446, A C T N 2, D 1 S 547, D 1 S 1609, and D 1 S 180. A few D N A markers are highlighted in the picture as follows: S P T A 1, C R P, A P O A 2, and A C T N 2. A caption reads, “D N A markers and a few genes (in blue) of known phenotypes can be used to determine the positions of genes.” Another caption pointing towards the A C T N 2 marker reads, “This gene encodes alpha-actinin, an actin-binding protein found in muscle cells. Mutation in this gene may be associated with muscular dystrophy.” The map on the right shows a picture of the condensed metaphase chromosome that is stained showing its banding pattern as dark and light in alternate manner as follows: 36 point 3 (light), 36 point 2 (dark), 36 point 1 (light) for D 1 S 434; 35 (dark), 34 point 3 (light), 34 point 2 (dark), 34

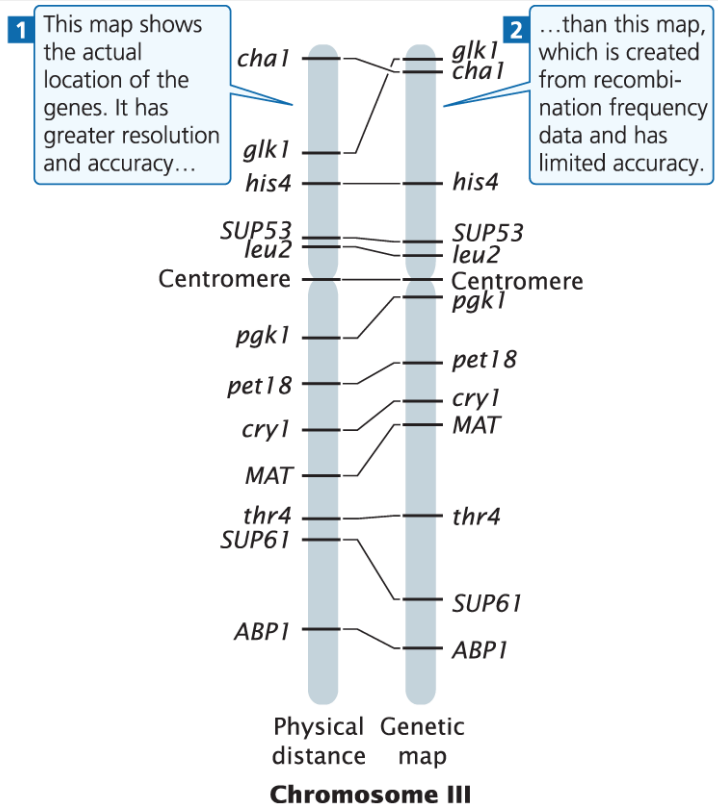
point 1 (light) for D 1 S 496; 33 (dark), 32 point 3 (light), 32 point 2 (dark), 32 point 1 (light) for D 1 S 209; 31 point 3 (dark), 31 point 2 (light), 31 point 1 (dark), 22 point 3 (light), 22 point 2 (dark), 22 point 1 (light); 21 (dark), 13 point 3 (light), 13 point 2 (dark), 13 point 1 (light) for D 1 S 221; 12 (dark), 11 (dark), 11 (dark), 12 (light), 21 point 1 (light), 21 point 2 (dark); 21 point 3 (light), 22 (dark), 23 (light), 24 (dark), 25 (light), 31 (dark), 32 point 1 (light), 32 point 2 (dark) for D 1 S 412; 32 point 3 (light), 41 (dark) for D 1 S 237; 42 point 1 (light), 42 point 2 (dark), 42 point 3 (light), 43 (dark), and 44 (light) for D 1 S 446. A caption reads, “Bands visible on a metaphase chromosome are numbered. The locations of some D N A markers relative to chromosome bands have been determined.”

For many years, genes could be detected only by observing their influence on a trait (the phenotype), and the construction of genetic maps was limited by the availability of single-locus traits that could be examined for evidence of recombination. Eventually, this limitation was overcome by the development of molecular techniques, such as the analysis of restriction sites, the polymerase chain reaction, and DNA sequencing (see Chapter 19), which are able to provide molecular markers that can be used to construct and refine genetic maps.

LIMITATIONS OF GENETIC MAPS

Genetic maps have several limitations, the first of which is resolution, or detail. The human genome includes 3.2 billion base pairs of DNA and has a total genetic distance of about 4000 cM, an average of 800,000 bp/cM. Even if at least one marker were present within each centiMorgan (which is unrealistic), their resolution in regard to the physical structure of the DNA would still be quite low.

In other words, the detail of a genetic map is very limited. A second problem with genetic maps is that they do not always accurately correspond to physical distances between genes. Genetic maps are based on rates of crossing over, which vary somewhat from one part of a chromosome to another; therefore, the distances on a genetic map are approximations of real physical distances along a chromosome. Figure 20.2 compares the genetic map of chromosome III of yeast with a physical map determined by DNA sequencing. There are some discrepancies in the distances between genes and even in the positions of some genes. In spite of these limitations, genetic maps have been critical to the development of physical maps and the sequencing of whole genomes.



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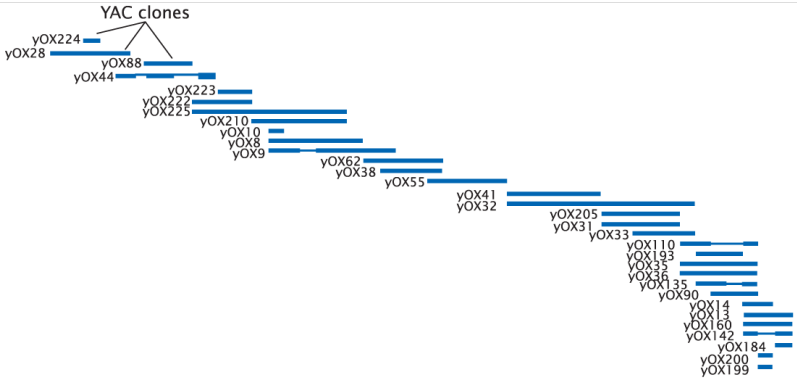
20.2 Genetic and physical maps may differ in relative distances between, and even in the positions of, genes on a chromosome. Genetic and physical maps of yeast chromosome III reveal such differences.

Description
The genes on the physical distance map from top to bottom are as follows: c h a 1, g l k 1, his 4, S U P 53, leu 2, centromere, p g k 1, pet 18, cry 1, M A T, t h r, 4, S U P 61, and A B P 1. A caption pointing towards this map reads, “This map shows the actual location of the genes. It has greater resolution and accuracy ellipsis.” The genes on the genetic map from top to bottom are as follows: g l k 1, his 4, S U P 53, leu 2, centromere, p g k 1, pet 18, cry 1, M A T, t h r 4, S U P 61, A B P 1. A caption pointing towards this map reads, “ellipsis than this map, which is created from recombination frequency data and has limited accuracy.”

Physical Maps

Physical maps are based on the direct analysis of DNA, and they place genes in relation to distances measured in number of base pairs, kilobases, or megabases. A common type of physical map is one that connects isolated pieces of genomic DNA that have been cloned in bacteria or yeast (**Figure 20.3**). Physical maps generally have higher resolution and are more accurate than genetic maps.



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20.3 Physical maps are often used to determine the order of cloned DNA fragments. Shown here is a part of a physical map of a set of overlapping cloned DNA fragments from one end of the human Y chromosome.

Description

The yac D N A fragments are labeled small y big O big X, then a number as follows: y OX 224, y OX 28, y OX 88, y OX 44, y OX 223, y OX 222, y OX 225, y OX 210, y OX 10, y OX 8, y OX 9, y OX 62, y OX 38, y OX 55, y OX 41, y OX 32, y OX 205, y OX 31, y OX 33, y OX 110, y OX 193, y OX 35, y OX 36, y OX 135, y OX 90, y OX 14, y OX 13, y OX 160, y OX 142, y OX 184, y OX2 00, and y OX 199.

One of the techniques that has been used in the past for creating physical maps is restriction mapping, which determines the positions of restriction sites in DNA. When a piece of DNA is cut (digested) with a restriction enzyme and the fragments are separated by gel electrophoresis, the number of restriction sites in the DNA and the distances between them can be determined by the number and positions of bands on the gel (see p. 578 in Chapter 19). However, this information does not tell us the order or the precise locations of the restriction sites. To map restriction sites, a sample of the DNA is cut with one restriction enzyme, and another sample

of the same DNA is cut with a different restriction enzyme. A third sample is cut with both restriction enzymes together (a double digest). The DNA fragments produced by these restriction digests are then separated by gel electrophoresis, and their sizes are compared. Overlap in the sizes of the fragments produced by the digests can be used to position the restriction sites on the original DNA molecule (see the Worked Problem at the end of this chapter). Most restriction mapping is done with several restriction enzymes, used alone and in various combinations, producing many restriction fragments. With long pieces of DNA (greater than 30 kb), computer programs are used to determine the restriction maps, and restriction mapping may be facilitated by labeling one end of a large DNA fragment with a chemical tag or by identifying the end with the use of a probe.

Physical maps, such as restriction maps of DNA fragments or even whole chromosomes, were often used in the past for genomic analysis. These lengthy maps are created by combining maps of shorter, overlapping DNA fragments. A number of additional techniques exist for creating physical maps: determining the positions of short, unique sequences of DNA on a chromosome; in situ hybridization, by which markers can be visually mapped to locations on chromosomes; and DNA sequencing.

CONCEPTS

Both genetic and physical maps provide information about the relative positions of and distances between genes, molecular markers, and chromosome segments. Genetic maps are based on rates of recombination and are measured in recombination frequencies

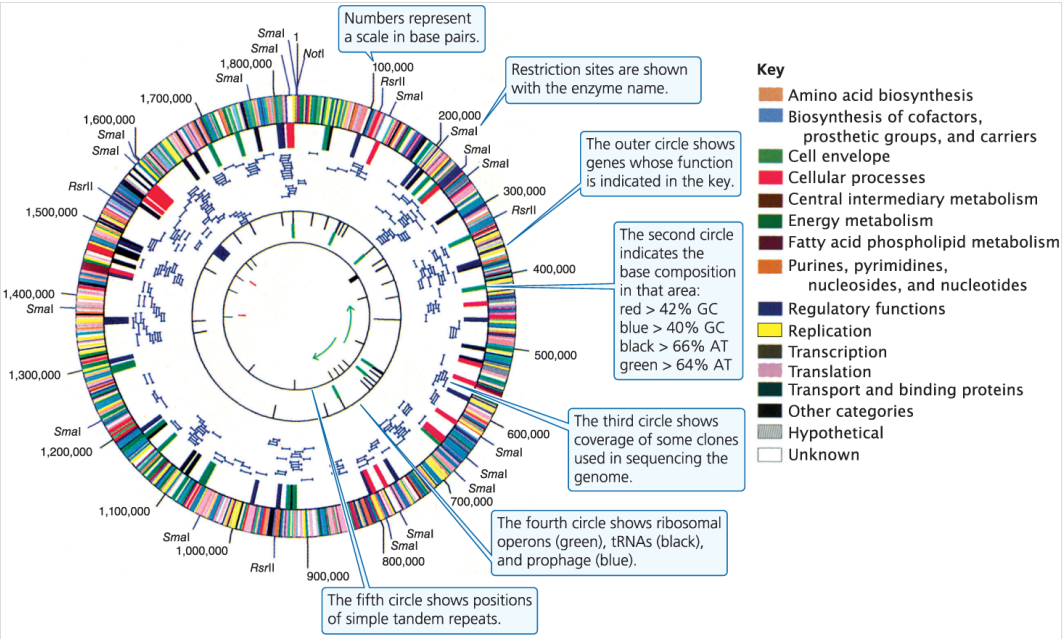
(centiMorgans or map units). Physical maps are based on physical distances and are measured in base pairs.

✔ CONCEPT CHECK 1

What are some of the limitations of genetic maps?

Sequencing an Entire Genome

The first genomes to be sequenced were small virus genomes. The genome of bacteriophage λ, consisting of 49,000 bp, was completed in 1982. In 1995, the first genome of a free-living organism (*Haemophilus influenzae*) was sequenced by Craig Venter and Claire Fraser of The Institute for Genomic Research (TIGR) and Hamilton Smith of Johns Hopkins University. This bacterium has a small genome of 1.8 million base pairs (**Figure 20.4**). By 1996, the genome of the first eukaryotic organism (yeast) had been determined, followed by the genomes of *Escherichia coli* (1997), *Caenorhabditis elegans* (1998), *Drosophila melanogaster* (2000), and *Arabidopsis thaliana* (2000). Sequencing of the human genome was largely completed in the spring of 2003.



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20.4 The bacterium *Haemophilus influenzae* was the first free-living organism to be sequenced. [From R. D. Fleischmann et al., Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd, *Science* 269(5223): 496–512, 1995. Reprinted with permission from AAAS. Scan courtesy of TIGR, permission conveyed through Copyright Clearance Center, Inc.]

Description

The map has 5 concentric circular elements. The outermost circle is labeled at the top position as base pair 1 and proceeds clockwise ending at base pair 1,800,000. Enzyme cleavage sites are indicated, and the genes regions involved in different processes (amino acid biosynthesis, replication, et cetera) are depicted by different colors. In clockwise direction, the sites are as follows: S m a 1, N o t 1, R s r 2, S m a 1, S m a 1, S m a 1, R s r 2, S m a 1, S m a 1, S m a 1, S m a 1, S m a 1, R s r 2, S m a 1, S m a 1, S m a 1, R s r 2, S m a 1, S m a 1, S m a 1, S m a 1, and S m a 1. The second circle indicates base composition. The second circle shows colors in clockwise direction as follows: red, green, black, blue, red, blue, black, green, green, red, black, green, blue, green, red, blue, black, black, red, red, blue, red, red, green, blue, red, red, blue, green, black, green, blue, blue, green, black, black, green, black, green, blue, green, blue, blue, blue, red, black, black, blue, black, black, red, red, black, black, green, green, black, and blue. The third circle shows the clones used to sequence the genome. The fourth circle shows ribosomal operons in green, t-R N A's in black, and prophage in blue. The fifth and last inner circle

shows the location of tandem repeats. Captions in the illustration are as follows: Numbers represent a scale in base pairs. Restriction sites are shown with the enzyme name. The outer circle shows genes whose function is indicated in the key. The second circle indicates the base composition in that area: red is greater than 42 percent G C, blue is greater than 40 percent G C, black is greater than 66 percent A T, and green is greater than 64 percent A T. The third circle shows coverage of some clones used in sequencing the genome. The fourth circle shows ribosomal operons (green), t R N A‘s (black), and prophage (blue). The ¬fifth circle shows positions of simple tandem repeats.” A key at the right describes the color used for various biomolecules or compounds as follows:

Mud brown: Amino acid biosynthesis.

Light blue: Biosynthesis of cofactors, prosthetic groups, and carriers.

Light green: Cell envelope.

Pink: Cellular processes.

Dark brown: Central intermediary metabolism.

Dark green: Energy metabolism.

Brown: Fatty acid phospholipid metabolism.

Light brown: Purines, pyrimidines, nucleosides, and nucleotides.

Dark blue: Regulatory functions.

Yellow: Replication.

Green: Transport and binding proteins.

Violet: Translation.

Grey: Transcription.

Black: Other categories.

Blue dots: Hypothetical.

White: Unknown.

The ultimate goal of structural genomics is to determine the ordered nucleotide sequences of entire genomes of organisms, providing the sequence information that is used to answer other questions. In Chapter 19, we considered some of the methods used to sequence fragments of DNA. The main obstacle to sequencing a whole genome is the immense size of most genomes. Bacterial genomes are usually at least several million base pairs long; many eukaryotic genomes are billions of base pairs long and are distributed among dozens of chromosomes. Furthermore, for technical reasons, sequencing cannot begin at one end of a chromosome and continue straight through to the other end; only small fragments of DNA—typically 500 to 700 nucleotides—are usually sequenced at one time. Therefore, determining the sequence for an entire genome requires that the DNA be broken into thousands or millions of small overlapping fragments that can then be sequenced. The difficulty lies in putting these short sequences back together in the correct order. Two different approaches have been used to assemble the sequenced fragments into a complete genome: map-based sequencing and whole-genome shotgun sequencing. Here we consider these two approaches in the context of the Human Genome Project, in which both approaches were used.

The Human Genome Project

By 1980, methods for mapping and sequencing DNA fragments had been sufficiently developed that geneticists began seriously

proposing that the entire human genome could be sequenced. An international collaboration was planned to undertake the Human Genome Project; initial estimates suggested that 15 years and $3 billion would be required to accomplish the task.

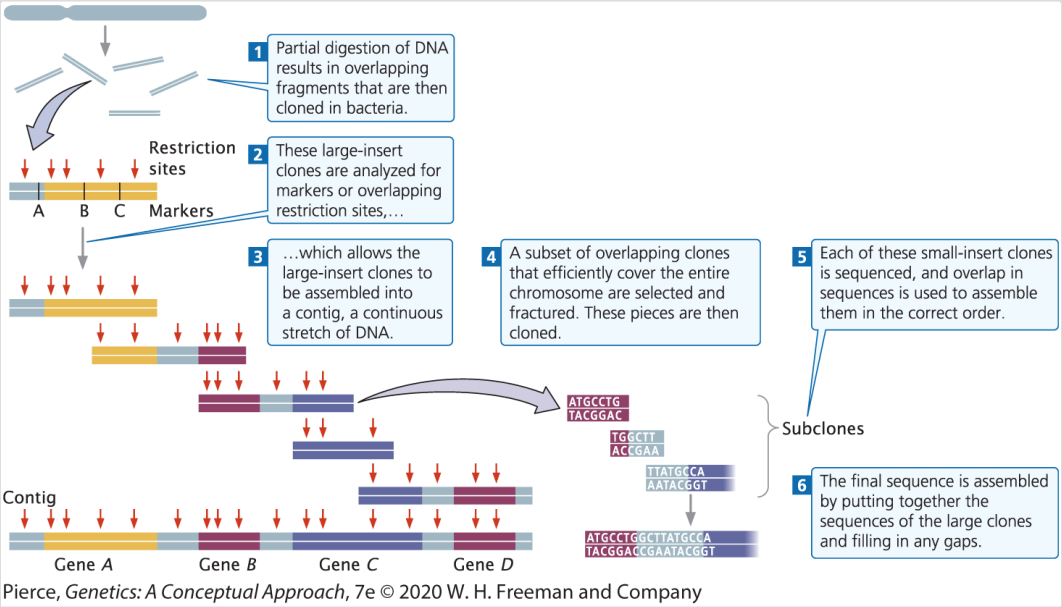
The Human Genome Project officially began in October 1990. Initial efforts focused on developing new and automated methods for cloning and sequencing DNA and on generating detailed physical and genetic maps of the human genome. The effort was a public project consisting of international collaboration among the 20 research groups and hundreds of individual researchers who formed the International Human Genome Sequencing Consortium. This group used a map-based strategy for sequencing the human genome.

MAP-BASED SEQUENCING

In **map-based sequencing**, short sequenced fragments are assembled into a whole-genome sequence by first creating detailed genetic and physical maps of the genome, which provide known locations of genetic markers (restriction sites, genes, or known DNA sequences) at regularly spaced intervals along each chromosome. These markers are later used to help align the short, sequenced fragments in their correct order.

Each chromosome (or sometimes the entire genome) is cut up by partial digestion with restriction enzymes (**Figure 20.5**), producing a large set of overlapping DNA fragments, which are then cloned with

the use of cosmids, yeast artificial chromosomes (YACs), or bacterial artificial chromosomes (BACs; see Section 19.3).



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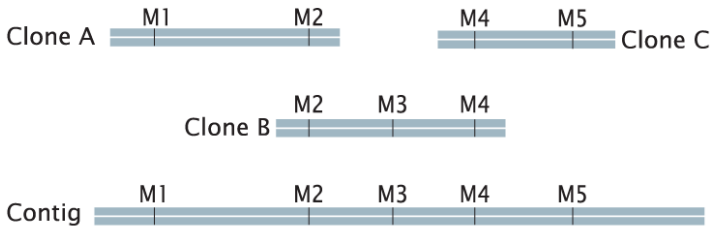
20.5 Map-based approaches to whole-genome sequencing rely on detailed genetic and physical maps to align sequenced fragments.

Description

A chromosome is shown, which is then digested and produced fragments. One of these fragments shows A B C markers and restriction sites. Then, some fragments are shown lined up at overlapping regions. Subclones are shown with the following template sequence with complementary sequence: 1, A T G C C T G; 2, T G G C T T; 3, T T A T G C C A. All these three subclones lead to a D N A with sequence: A T G C C T G G C T T A T G C C A. The other strand has a complementary sequence. The captions in the illustration are as follows: “Partial digestion of D N A results in overlapping fragments that are then cloned in bacteria. These large-insert clones are analyzed for markers or overlapping restriction sites, which allows the large-insert clones to be assembled into a contig, a continuous stretch of D N A. A subset of overlapping clones that efficiently cover the entire chromosome are selected and fractured. These pieces are then cloned. Each of these small-insert clones is sequenced, and overlap in sequences is used to assemble them in the correct order. The ¬final sequence is assembled by putting together the sequences of the large clones and fi¬lling in any gaps.”

Next, these large-insert clones are put together in their correct order on the chromosome (see Figure 20.5) using a high-density map of genetic markers (variable sequences that can be detected). A complementary DNA probe is made for each genetic marker, and a library of the large-insert clones is screened with the probe, which hybridizes to any colony containing a clone with the marker. The library is then screened for neighboring markers. Because the clones are much larger than the markers used as probes, some clones will have more than one marker. For example, clone A might have markers M1 and M2; clone B markers M2, M3, and M4; and clone C markers M4 and M5. Such a result would indicate that these clones contain areas of overlap, as shown here:



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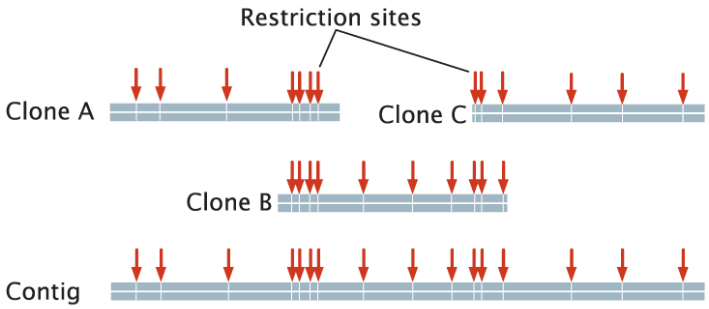
Description
Clone A has genes M 1 and M 2. Clone B has genes M 2, M 3, and M 4. Clone C has genes M 4 and M 5. The contig below shows the genes in the following sequence: M 1, M 2, M 3, M 4, and M 5.

A set of two or more overlapping DNA fragments that form a contiguous stretch of DNA is called a **contig**. This approach was used

in 1993 to create a contig consisting of 196 overlapping YAC clones (see Figure 20.3) of the human Y chromosome.

The order of clones can also be determined without the use of preexisting genetic maps. For example, each clone can be cut with a series of restriction enzymes and the resulting fragments then separated by gel electrophoresis. A computer program is then used to examine the restriction patterns of all the clones and look for areas of overlap. The overlap is then used to arrange the clones in order, as shown here:



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Description
Clone A has 7 restriction sites. Clone B has 10 restriction sites. Clone C has 6 restriction sites. The entire contig has 16 restriction sites.

Other genetic markers can be used to help position contigs along the chromosome. **› TRY PROBLEM 27**

When the large-insert clones have been assembled in the correct order on the chromosome, a subset of overlapping clones that

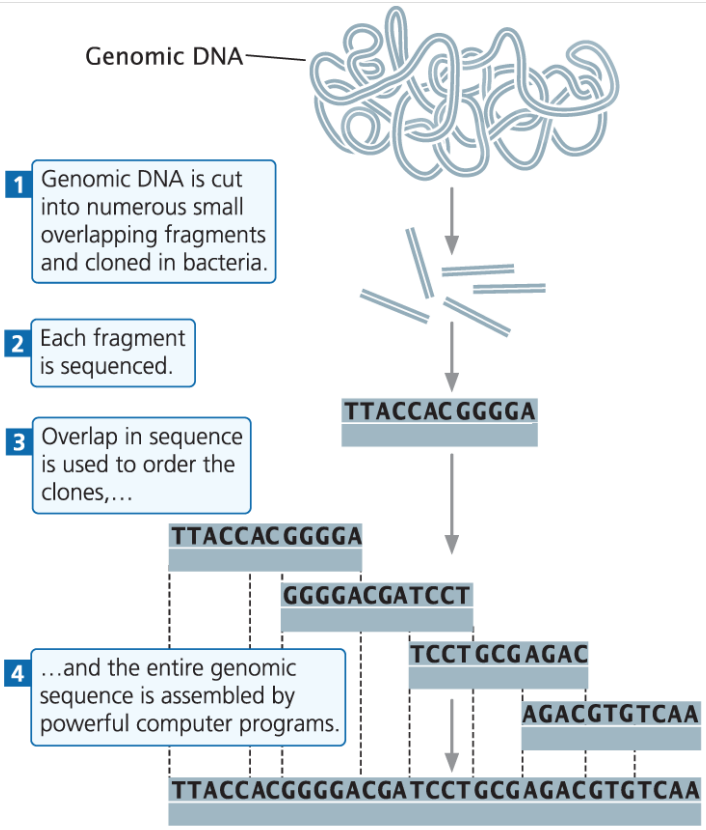
efficiently cover the entire chromosome can be chosen for sequencing; the goal is to select the minimum number of clones that is necessary to represent the chromosome. Each of the selected large-insert clones is fractured into smaller overlapping fragments, which are themselves cloned (see Figure 20.5) and sequenced. The sequences of the small-insert clones are examined for overlap, which allows them to be correctly assembled to give the sequence of the large-insert clones. Enough overlapping small-insert clones are usually sequenced to ensure that the entire genome is sequenced several times. Finally, the whole genome is assembled by putting together the sequences of all overlapping contigs (see Figure 20.5). Often, gaps in the genome sequence still exist and must be filled in by using other methods.

The International Human Genome Sequencing Consortium used a map-based approach to sequencing the human genome. Many copies of the human genome were cut up into fragments of about 150,000 bp each, which were inserted into bacterial artificial chromosomes. Yeast artificial chromosomes and cosmids were used in early stages of the project but did not prove to be as stable as the BAC clones, although YAC clones were instrumental in putting together some of the larger contigs. Restriction fingerprints and other genetic markers were used to assemble the BAC clones into contigs, which were positioned on the chromosomes with the use of genetic markers and probes. The individual BAC clones were then sheared into smaller overlapping fragments and sequenced, and the

whole genome was assembled by putting together the sequence of the BAC clones.

WHOLE-GENOME SHOTGUN SEQUENCING

In 1998, Craig Venter announced that he would lead a company called Celera Genomics in a private effort to sequence the human genome. He proposed using a shotgun sequencing approach, which he suggested would be quicker than the map-based approach employed by the International Human Genome Sequencing Consortium. In **whole-genome shotgun sequencing** (**Figure 20.6**), small-insert clones are prepared directly from genomic DNA and sequenced. Powerful computer programs then assemble the entire genome by examining overlap among the small-insert clones. The requirement for overlap means that most of the genome must be sequenced many times. The average number of times a nucleotide in the genome is sequenced is called the **sequencing coverage**. For example, 10× coverage means that an average nucleotide in the genome has been sequenced 10 times. In general, genome sequences with more coverage are considered more accurate.



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20.6 Whole-genome shotgun sequencing uses sequence overlap to align sequenced fragments.

Description

The illustration starts with a genomic D N A, which is then digested into fragments. The sequence of the upper strand of one fragment is shown as T T A C C A C G G G G A. It leads to four fragments lined up at overlapping regions as follows: 1, T T A C C A C G G G G A; 2, G G G G A C G A T C C T; 3, T C C T G C G A G A C; and 4, A G A C G T G T C A A. The sequence of the entire contig is as follows: T T A C C A C G G G G A C G A T C C T G C G A G A C G T G T C A A. The captions in the illustration read as follows: “Genomic D N A is cut into numerous small overlapping fragments and cloned in bacteria. Each fragment is sequenced. Overlap in sequence is used to order the clones, and the entire genomic sequence is assembled by powerful computer programs.”

Shotgun sequencing was initially used for assembling small genomes, such as those of bacteria. When Venter proposed the use of this approach for sequencing the human genome, it was not at all clear that the approach could successfully assemble a complex genome consisting of billions of base pairs. Today, virtually all genomes are sequenced using the whole-genome shotgun approach. For more information on how the human genome was sequenced, see (A) **Animation 20.1**.

CONCEPTS

Sequencing a genome requires breaking it up into small overlapping fragments whose DNA sequences can be determined in a sequencing reaction. In map-based sequencing, sequenced fragments are ordered into the final genome sequence with the use of genetic and physical maps. In whole-genome shotgun sequencing, the genome is assembled by means of overlap in the sequences of small fragments.

✔ CONCEPT CHECK 2

A contig is

a. a set of molecular markers used in gene mapping.
b. a set of overlapping fragments that form a continuous stretch of DNA.
c. a set of fragments generated by a restriction enzyme.
d. a small DNA fragment used in sequencing.

RESULTS AND IMPLICATIONS OF THE HUMAN GENOME PROJECT

In the summer of 2000, both public and private sequencing projects announced the completion of a rough draft that included most of the sequence of the human genome, five years ahead of schedule (Figure 20.7). The human genome sequence was declared completed in the spring of 2003, although some gaps still remain. For most chromosomes, the finished sequence is 99.999% accurate, with less than one base-pair error per 100,000 bp, an accuracy rate 10 times that of the initial goal.

Alex Wong/Newsmakers/Getty Images.

20.7 Craig Venter (left), President of Celera Genomics, and Francis Collins (right), Director of the National Human Genome Research Institute, NIH, announce the completion of a rough draft of the human genome at a press conference in Washington, D.C., on June 26, 2000.

With the first human genome determined, sequencing additional human genomes is much easier. It is now possible to sequence an entire human genome in a few hours, and genomes from thousands of different people have now been sequenced. The cost of sequencing a complete human genome has also dropped

dramatically and will continue to fall as sequencing technology improves.

The availability of the complete sequence of the human genome is proving to be of great benefit. The sequence has provided tools for detecting and mapping genetic variants across the human genome, greatly facilitating gene mapping in humans. For example, as we will see shortly, several million sites at which people differ in a single nucleotide (called single-nucleotide polymorphisms) have now been identified, and these sites are being used in genome-wide association studies to locate genes that affect diseases and traits in humans. The sequence is also providing important information about development and many basic cellular processes.

Next-generation and third-generation sequencing techniques that allow rapid and inexpensive sequencing of genomic DNA (see Section 19.5) are being used to address fundamental questions in many areas. For example, the genomes of a number of cancer cells have now been completely sequenced and compared with the sequences of healthy cells from the same person, allowing complete determination of all the mutations that lead to tumor formation and cancer progression. It is now possible to sequence the genomes of single cells, providing information about somatic mutations and how, for example, cells within a tumor change over time. The complete genome of an unborn baby has been sequenced from fetal DNA isolated from its mother's blood.

Researchers have reported the analysis of genomes from over 2600 Icelanders, providing a spectacular picture of the genetic composition of a nation. The 1000 Genomes Project, completed in 2015, sequenced and compared the genomes of 2504 individuals from 26 populations in Africa, East Asia, Europe, South Asia, and the Americas, with the goal of detecting most of the common variations that exist in the human species. Sequencing of the complete genomes of parents and their children has allowed a direct estimate of mutation rates.

Genomes have been sequenced from DNA extracted from the fossils of long-extinct animals, such as a 700,000-year-old Pleistocene horse. DNA has been isolated and genomes sequenced from the bones of ancient humans, including Neanderthals and Denisovans (a little-known group of humans that appear to be closely related to Neanderthals; see introduction to Chapter 10). Comparisons of the modern human genome with the genomes of these and other species are adding to our understanding of human evolution. For example, research now suggests that the genomes of Europeans and Asians consist of 1% to 4% Neanderthal DNA, which was transferred to modern humans when interbreeding took place between the two species.

Along with the many potential benefits of having complete human sequence information, there are concerns about it being misused. With the knowledge gained from genome sequencing, many more genes for diseases, disorders, and behavioral and physical traits have

been identified, increasing the number of genetic tests that can be performed to make predictions about the future phenotype and health of a person. There is concern that information from genetic testing might be used to discriminate against people who are carriers of disease-causing genes or who might be at risk for some future disease. This matter has been addressed to some extent in the United States with the passage of the Genetic Information Nondiscrimination Act (see Section 6.4), which prohibits health insurers and employers from using genetic information to make decisions about health-insurance coverage and employment (although it does not apply to life, disability, or long-term care insurance). Questions also arise about who should have access to a person's genome sequence. What about relatives, who have similar genomes and might also be at risk for some of the same diseases? There are also questions about the use of this information to select for specific traits in future offspring. All these concerns are legitimate and must be addressed if we are to use the information from genome sequencing responsibly.

THINK-PAIR-SHARE Question 1

CONCEPTS

The Human Genome Project was an effort to sequence the entire human genome. Rough drafts of the sequence were completed by two competing teams in 2000. The entire sequence was published in 2003, although gaps in the sequence remain. The ability to rapidly sequence human genomes raises a number of ethical questions.

What Exactly Is the Human Genome?

As we have seen, the human genome sequence was determined first as rough drafts in 2000 and then in its "final" form in 2003. This sequence has been of immense importance in research, medicine, and biotechnology. However, geneticist Ken Weiss has argued that what people refer to as "the human genome" does not actually exist: there is not a single genome that represents the human species. What was determined by the Human Genome Project is a *reference* genome, which was based on DNA donated from not one but several different individuals whose identities are kept confidential for ethical reasons. In reality, there are billions of human genomes; every person's genome is likely to be unique, differing at least a little from every other person's genome. At millions of nucleotide positions across the genome, people vary in the particular base that occurs; some people, for example, possess a T, whereas others possess a G. Some people even have extensive segments of DNA (called structural variants) that are missing, duplicated, or rearranged. Furthermore, we know that there is not even a single genome sequence for a person; research has demonstrated that individual cells within the same person vary in their DNA sequences due to somatic mutations.

It's important to keep in mind that the official human reference genome is not the ancestral sequence for all humans, nor even the most common genome sequence among humans. Rather, it is an

arbitrary sequence, based on the genetic idiosyncrasies of those individuals whose DNA was sequenced in the Human Genome Project. And even the reference genome keeps changing, as corrections and better sequences for problematic regions are obtained.

Among nonhuman species, especially in bacteria, the problem of a single reference genome becomes even more significant. In many bacteria, different strains of the same species not only harbor variation within genes but may even have different sets of genes. This has led to the concept of a *pangenome*, defined as the entire set of genes possessed by all members of a particular species. As sequencing becomes easier and less expensive, researchers are focusing more effort on understanding the diversity that exists within genomes.

THINK-PAIR-SHARE Question 2

Single-Nucleotide Polymorphisms

Genome sequencing has identified a number of sites where humans differ in their genome sequences. Imagine that you are riding in an elevator with a random stranger. How much of your genome do you have in common with this person? Studies of variation in the human genome indicate that you and the stranger will be identical at about

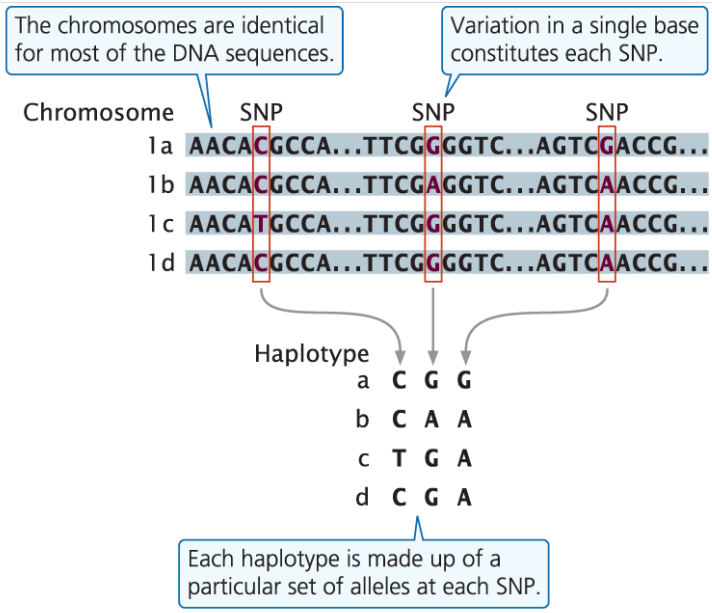
99.9% of your DNA sequences (not including structural variations such as duplications, deletions, copy-number variants, and other types of variations; see Chapters 8 and 18). The difference between you and the stranger is very small in *relative* terms, but because the human genome is so large (3.2 billion base pairs), you and the stranger will be different at more than 3 million base pairs of your genomic DNA. These differences are what make each of us unique, and they greatly affect our physical features, our health, and possibly even our intelligence and personality.

A site in the genome where individual members of a species differ in a single base pair is called a **single-nucleotide polymorphism** (**SNP**, pronounced "snip"). To be considered a SNP, a variant must reach a frequency of at least 1% in a population; thus, very rare variations are not usually considered SNPs. Arising through mutation, SNPs are inherited as allelic variants in the same way as alleles that produce phenotypic differences (such as blood types), although SNPs often do not produce phenotypic differences. Single-nucleotide polymorphisms are numerous and are present throughout genomes. In a comparison of the same chromosome from two different people, a SNP can be found approximately every 1000 bp.

THINK-PAIR-SHARE Question 3

HAPLOTYPES AND LINKAGE DISEQUILIBRIUM

Most SNPs present within a population arose once from a single mutation that occurred on a particular chromosome in a particular individual and subsequently spread throughout the population. Thus, each SNP is initially associated with other SNPs (as well as with other types of genetic variants or alleles) that were present on the particular chromosome on which the mutation arose. The specific set of SNPs and other genetic variants observed on a single chromosome or part of a chromosome is called a **haplotype** (**Figure 20.8**). SNPs within a haplotype are physically linked and therefore tend to be inherited together. New haplotypes can arise through mutation or crossing over, which breaks up the particular set of SNPs in a haplotype. Because the rate of crossing over is proportional to the physical distance between genes, SNPs and other genetic variants that are located close together on a chromosome will be strongly associated as haplotypes. The nonrandom association between genetic variants within a haplotype is called *linkage disequilibrium* (see Section 7.3). Because the SNPs in a haplotype are inherited together, a haplotype consisting of thousands of SNPs can be identified with the use of only a few SNPs. The few SNPs used to identify a haplotype are called **tag-SNPs**. There are about 10 million SNPs in the human population, but because of linkage disequilibrium, these SNPs can be grouped into a much smaller number of haplotypes. Therefore, a relatively small number of SNPs—perhaps only 100,000—can be used to identify most of the haplotypes in humans.



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20.8 A haplotype is a specific set of SNPs and other genetic variants observed on a single chromosome or part of a chromosome. Chromosomes 1a, 1b, 1c, and 1d represent different copies of a chromosome that might be found in a population.

Description

Chromosome 1 a shows the following sequence in three sets: A A C A C G C C A ellipsis T T C G G G G T C ellipsis A G T C G A C C G. Chromosome 1 b shows the following sequence in three sets: A A C A C G C C A ellipsis T T C G A G G T C ellipsis A G T C A A C C G. Chromosome 1 c shows the following sequence in three sets: A A C A T G C C A ellipsis T T C G G G G T C ellipsis A G T C A A C C G. Chromosome 1 d shows the following sequence in three sets: A A C A C G C C A ellipsis T T C G G G G T C ellipsis A G T C A A C C G. A caption pointing towards the first chromosome reads, “The chromosomes are identical for most of the D N A sequences.” Three vertical boxes drawn in each set for four chromosomes represent S N P’s as follows: C C T C, G A G G, and G A A A. A caption pointing towards the S N P reads, “Variation in a single base constitutes each S N P.” These S N P’s are then written vertically side by side labeled haplotype. A caption reads, “Each haplotype is made up of a particular set of alleles at each S N P.”

THE USE OF SINGLE-NUCLEOTIDE POLYMORPHISMS

Because of their variability and widespread occurrence throughout the genome, SNPs are valuable as markers in linkage studies. When a SNP is physically close to a disease-causing locus, it will tend to be inherited along with the disease-causing allele. Therefore, people with the disease will tend to have different SNPs than healthy people. A comparison between SNP haplotypes in people with a disease and those in healthy people can reveal the presence of genes that affect the disease; because the disease gene and the SNP are closely linked, the location of the disease-causing gene can be determined from the locations of associated SNPs.

An international effort, called the International HapMap Project, cataloged and mapped SNPs and other genetic variants that could be used to identify common haplotypes in human populations for use in linkage and family studies. The project cataloged a total of 4.6 million SNPs from human populations. These SNPs are spread over all 22 human autosomes, as well as the X and Y chromosomes. The study found alleles from most of the common SNPs in four ethnic groups (African, Japanese, Chinese, and European), although the frequencies of the alleles vary considerably among human populations. The greatest genetic diversity of SNPs is found within Africans, which is consistent with many other studies that suggest that humans first evolved in Africa.

Data from the HapMap Project have been used to provide important information about the function and evolution of the human genome. For example, studies of linkage disequilibrium with the use of SNPs have determined that recombination does not take place randomly across the chromosomes; there are numerous recombination hotspots where more recombination takes place than expected merely by chance. The distribution of SNPs is also providing information about regions of the human genome that have evolved quickly in the recent past, providing clues to how humans have responded to natural selection.

GENOME-WIDE ASSOCIATION STUDIES

Many common diseases are caused by complex interactions among multiple genes. The availability of SNPs has greatly facilitated the search for these genes. **Genome-wide association studies** use numerous SNPs scattered across the genome to find genes of interest. Soon after the completion of the first phase of the HapMap Project, researchers used its SNP data to conduct a genome-wide association study of age-related macular degeneration, one of the leading causes of blindness among the elderly. In this study, researchers genotyped 96 people with macular degeneration and 50 unaffected people for more than 100,000 SNPs that blanketed the genome. The study revealed a strong association between the disease and a gene on chromosome 1 that encodes complement factor H, which has a role in immune function. This finding suggests that macular degeneration might be treatable with drugs that affect

complement proteins. Another study uncovered a major gene associated with Crohn disease, a common inflammatory disorder of the gastrointestinal tract. Other studies using genome-wide scans of SNPs have identified genes that contribute to heart disease, bone density, prostate cancer, diabetes, and many more medical conditions.

In one application of SNPs for finding disease associations, researchers genotyped 17,000 people in the United Kingdom for 500,000 SNPs in 2007. They detected strong associations between 24 genes or chromosome segments and the incidence of seven common diseases: coronary artery disease, Crohn disease, rheumatoid arthritis, bipolar disorder, hypertension, and two types of diabetes. The importance of this study is its demonstration that genome-wide association studies using SNPs could successfully locate genes that contribute to complex diseases caused by multiple genetic and environmental factors.

Within the past few years, SNPs have been used in genome-wide association studies to successfully locate genes that influence many additional traits, such as height, body mass index, the age of puberty and menopause in women, variation in facial features, skin pigmentation, eye color, glaucoma, and even susceptibility to infectious diseases such as meningococcal disease and tuberculosis. Unfortunately, the genes identified often explain only a modest proportion of the genetic influence on the trait. For example, one huge genome-wide association study combined data from over

100,000 people in an attempt to locate genes encoding blood lipids that are involved in cardiovascular disease. Although the study identified 95 different loci associated with lipid traits, these genes corresponded to only 25%–30% of the total genetic variation in these traits.

The missing DNA sequences that encode the majority of the genetic variation in such traits—sometimes called the "dark matter of the genome"—have thus far remained largely undetected. The low percentage of variation explained by most current genome-wide association studies means that the genes identified are not, by themselves, useful predictors of the risk of inheriting the disease or trait. Nevertheless, the identification of specific genes that influence a disease or trait can lead to a better understanding of the biological processes that produce the phenotype.

CONCEPTS

Genome projects are collecting databases of nucleotides that vary among individual organisms (single-nucleotide polymorphisms, or SNPs). Collections of SNPs are used in genome-wide association studies to find genes that contribute to diseases and other phenotypes.

✔ CONCEPT CHECK 3

What did the HapMap Project accomplish?

Bioinformatics

Complete genome sequences have now been determined for numerous organisms, and many additional projects are under way. These studies are producing tremendous quantities of sequence data. Cataloging, storing, retrieving, and analyzing this huge data set are major challenges of modern genetics. **Bioinformatics** is an interdisciplinary field that combines molecular biology and computer science; it centers on the development of databases, computer-search algorithms, gene-prediction software, and other analytical tools that are used to make sense of DNA-, RNA-, and protein-sequence data. Bioinformatics develops and applies these tools to "mine the data"; that is, these tools extract the useful information from sequencing projects. The development and use of algorithms and computer software for analyzing sequence data have helped to make molecular biology a more quantitative field. The storage of sequence data in publicly available online databases enables scientists and students throughout the world to access this tremendous resource.

A number of databases have been established for the collection and analysis of DNA- and protein-sequence information (**Table 20.2**). Primary databases contain the sequence information, along with information that describes the source of the sequence and its determination. Secondary databases contain the results of analyses carried out on the primary sequence data, such as information about

particular sequence patterns, variations, mutations, and evolutionary relationships.

TABLE 20.2 Some widely used DNA, RNA, and protein databases

Database	Description
GenBank	Public database of known DNA sequences
EMBL (European Molecular Biology Laboratory) nucleotide sequence database	Database of DNA and RNA sequences
DDBJ (DNA Database of Japan)	Database of DNA sequences
IGSR (The International Genome Sample Resource)	Public database of known DNA variations in human populations, including data from the HapMap Project and the 1000 Genomes Project
ArrayExpress	Database of data from functional genomics

	experiments
miRNA	Database of microRNA sequences
GEO (Gene Expression Omnibus)	Public database of microarrays, next-generation sequencing, and other forms of genomics data
UKB (UK Biobank)	Database of over 500,000 participants from the United Kingdom with extensive phenotypic and genotypic details, including physical measures, health outcomes, lifestyle variables, and 800,000 genetic markers
SwissProt	Protein sequence database
PDB (Protein Data Bank)	Archive of information about the 3D structures of large biological molecules, including proteins and nucleic acids
BioGRID (Biological General Repository for Interaction Datasets)	Database of genetic and protein interactions

After a genome has been sequenced, one of the first tasks is to identify potential genes within the sequence. Unfortunately, there are no universal characteristics that mark the beginning and end of a gene. The enormous amount of DNA in a genome and the complexities of gene structure make finding genes within a sequence a difficult task. Computer programs have been developed to look for specific sequences in DNA that are associated with certain genes. There are two general approaches to finding genes. The *ab initio* approach scans the sequence, looking for features that are usually found within a gene. For example, protein-encoding genes are characterized by an open reading frame, which includes a start codon and a stop codon in the same reading frame. Specific sequences mark the splice sites at the beginnings and ends of introns; other specific sequences are present in promoters immediately upstream of start codons. The comparative approach looks for similarity between a new sequence and the sequences of all known genes. If a match is found, then the new sequence is assumed to be a similar gene. Some of these computer programs are capable of examining databases of protein sequences to see if there is evidence that a potential gene is expressed.

It is important to note that the programs that have been developed to identify genes on the basis of DNA sequence are not perfect. Therefore, the numbers of genes reported in most genome-sequencing projects are estimates. The presence of multiple introns, alternative splicing, multiple copies of some genes, and much

noncoding DNA between genes makes accurate identification and counting of genes difficult.

After a gene has been identified, it must be **annotated**, which means linking its sequence information to other information about its function and expression, the protein it encodes, and similar genes in other species. There are a number of methods of probing a gene's function, which will be discussed in Section 20.2. Computer programs are available for determining whether similar sequences have already been found, either in the same species or in different species. The most widely used of these programs is the Basic Local Alignment Search Tool (BLAST). To conduct a BLAST search, a researcher submits a query sequence, and the program searches the database for any other sequences that have regions of high similarity to the query sequence. The program returns all sequences in the database that are similar, along with information about the degree of similarity and the significance of the match (how likely the similarity would be to occur by chance alone).

Additional databases contain information on sequence diversity: how and where a genome varies among individual organisms. The human genome has now been mapped for millions of SNPs, and information about the frequency of these variants in different populations is being collected. Additional databases have been developed to catalog all known mutations causing particular diseases; the Human Variome Project is an effort to collect and make available all genetic variations that affect human health. Important

information about the expression patterns of the thousands of genes found in genomes is also being compiled. ❯ **TRY** PROBLEM 30

CONCEPTS

Bioinformatics is an interdisciplinary field that combines molecular biology and computer science. It develops databases of DNA, RNA, and protein sequences, as well as tools for analyzing those sequences.

✔ CONCEPT CHECK 4

The *ab initio* approach finds genes by looking for

a. common sequences found in most genes.
b. similarity in sequence with known genes.
c. mRNA with the use of in situ hybridization.
d. mutant phenotypes.

Metagenomics

Advances in sequencing technology, which have made sequencing faster and less expensive, now provide the possibility of sequencing not just the genomes of individual species but the genomes of entire communities of organisms. **Metagenomics** is an emerging field in which the genome sequences of an entire group of organisms that inhabit a common environment are sampled and determined.

Thus far, metagenomics has been applied largely to microbial communities. It provides the ability to address two important issues: (1) the identification and study of microbes that cannot be cultured in the laboratory and (2) the study of the community structure of microorganisms.

Traditionally, bacteria have been studied by growing and analyzing them in the laboratory. However, many bacteria cannot be cultured with the use of laboratory techniques. Metagenomics analyzes microbial communities by extracting DNA from the environment, determining its sequences, and reconstructing community composition and function on the basis of those sequences. This technique allows the identification and genetic analysis of species that cannot be grown in the laboratory and have never been studied by traditional microbiological methods. The entire genomes of some dominant species have been reconstructed from environmental samples, providing scientists with a great deal of information on the biology of these microbes.

An early metagenomic study analyzed the microbial community found in acid drainage from a mine and determined that this community consisted of only a few dominant bacterial species. Another study, called the Global Ocean Sampling Expedition, followed the route of Darwin's voyage on H.M.S. *Beagle* in the 1800s. Scientists collected ocean samples and used metagenomic methods to determine the microbial communities they contained. In this study, scientists catalogued sequences for more than 6 million

proteins, including more than 1700 new protein families. Some important results have already emerged from metagenomic studies. Analyses of bacterial genomes found in ocean samples led to the discovery of proteorhodopsin proteins, which are light-driven proton pumps. Subsequent research demonstrated that these proteins are found in diverse microbial groups and are a major source of energy flux in the world's oceans.

Other metagenomic studies have examined the genes of bacteria that inhabit the human intestinal tract. These bacteria, along with those that inhabit the skin and other parts of the human body, are termed the human **microbiome**. The microbiome of a typical person probably includes as many bacterial cells as—or perhaps even more than—the number of human cells, although the number of bacteria will clearly vary from one person to the next. The National Microbiome Initiative, unveiled in 2016, is a project to advance scientific knowledge and understanding of microbiomes.

Research is demonstrating that the human microbiome plays an important role in human health. One study examined the gut microflora of obese and lean people. Two groups of bacteria are common in the human gut: Bacteroidetes and Firmicutes. Researchers discovered that obese people have relatively more Firmicutes than do lean people and that the proportion of Firmicutes decreases in obese people who lose weight on a low-calorie diet. These same results were observed in obese and lean mice. In an elegant experiment, researchers transferred bacteria

from obese to lean mice. Lean mice that received bacteria from obese mice extracted more calories from their food and stored more fat, suggesting that gut microflora might play some role in obesity.

Ecological communities have traditionally been characterized on the basis of the species they contain. Metagenomics affords the possibility of characterizing communities on the basis of their component genes, an approach that has been termed gene-centric. A gene-centric approach leads to new questions: Are certain types of genes more common in some communities than others? Are some genes essential for energy flow and nutrient recycling within a community? Because of the larger sizes of their genomes, eukaryotic communities have not yet been the focus of these approaches, but many researchers predict that they will be in the future.

CONCEPTS

Metagenomic studies examine the genomes of communities of organisms that inhabit a common environment. This approach has been applied to microbial communities and allows the composition and genetic makeup of a community to be determined without cultivating and isolating individual species. Metagenomic studies are a source of important new insights into microbial communities.

Synthetic Biology

Our ability to sequence and study whole genomes, coupled with our increased understanding of the genetic information required for basic biological processes, now provides the possibility of creating—entirely from scratch—novel organisms that have never before existed. **Synthetic biology** is a new field that seeks to design organisms that might provide useful functions, such as microbes that provide clean energy or break down toxic wastes.

Synthetic biologists have already mixed and matched parts from different organisms to synthesize microbes. In 2002, geneticists re-created the poliovirus by joining together pieces of DNA that were synthesized in the laboratory. Even more impressively, in 2010, Daniel Gibson and his colleagues synthesized, from scratch, the complete 1.08 million-base-pair genome of the bacterium *Mycoplasma mycoides*. They started with 1000 pieces of DNA that were synthesized in the laboratory and joined them together in successively larger pieces until they had assembled a complete copy of the genome. Within their synthetic genome, they included a set of DNA sequences that spelled out—in code—an email address, the names of the researchers who participated in the project, and several well-known quotations. Finally, the researchers transplanted the artificial genome into a cell of a different bacterial species, *Mycoplasma. capricolum*, whose original genome had been removed. The new cell then began expressing the traits specified by the synthetic genome.

Synthetic biology has also been extended to eukaryotic cells. In 2014, geneticists created a synthetic chromosome that successfully replaced a natural chromosome in yeast cells. By 2017, six artificial yeast chromosomes had been constructed, constituting almost 30% of the total yeast genome, and efforts are currently underway to engineer an entirely synthetic yeast genome. Another effort, called the Human Genome Project-Write (HGP-Write), has been proposed for synthesizing an entire human genome from scratch. However, current technologies are not capable of synthesizing such a large synthetic genome, and the idea has been controversial.

These types of experiments have raised a number of concerns. The ability to tailor-make novel genomes, and to mix and match parts from different organisms, creates the potential for the synthesis of dangerous microbes that might create ecological havoc if they escaped from the laboratory or that could be used in biological warfare or bioterrorism. Creating a synthetic human genome has raised concerns that such efforts might be used to artificially enhance human traits. Ongoing discussions among geneticists, ethicists, security experts, and politicians are addressing these concerns and whether synthetic genomes can be safely made and used.

THINK-PAIR-SHARE Questions 4 and 5

20.2 Functional Genomics Determines the Functions of Genes by Using Genomic Approaches

A genome sequence is, by itself, of limited use. Merely knowing the sequence of a genome would be like having a huge set of encyclopedias without being able to read: you could recognize the different letters, but the text would be meaningless. **Functional genomics** characterizes what sequences do—their function. The goals of functional genomics include the identification of all the RNA molecules transcribed from a genome, called the **transcriptome** of that genome, and all the proteins encoded by the genome, called the **proteome**. Functional genomics uses both bioinformatics and laboratory-based experimental approaches in its effort to define the functions of DNA sequences.

In Chapter 19, we considered several methods for identifying genes and assessing their functions, including in situ hybridization, experimental mutagenesis, and the use of transgenic animals and knockouts. Those methods can be applied to individual genes and can provide important information about the locations and functions of genetic information. In this section, we focus primarily on methods that can be applied to large numbers of genes simultaneously.

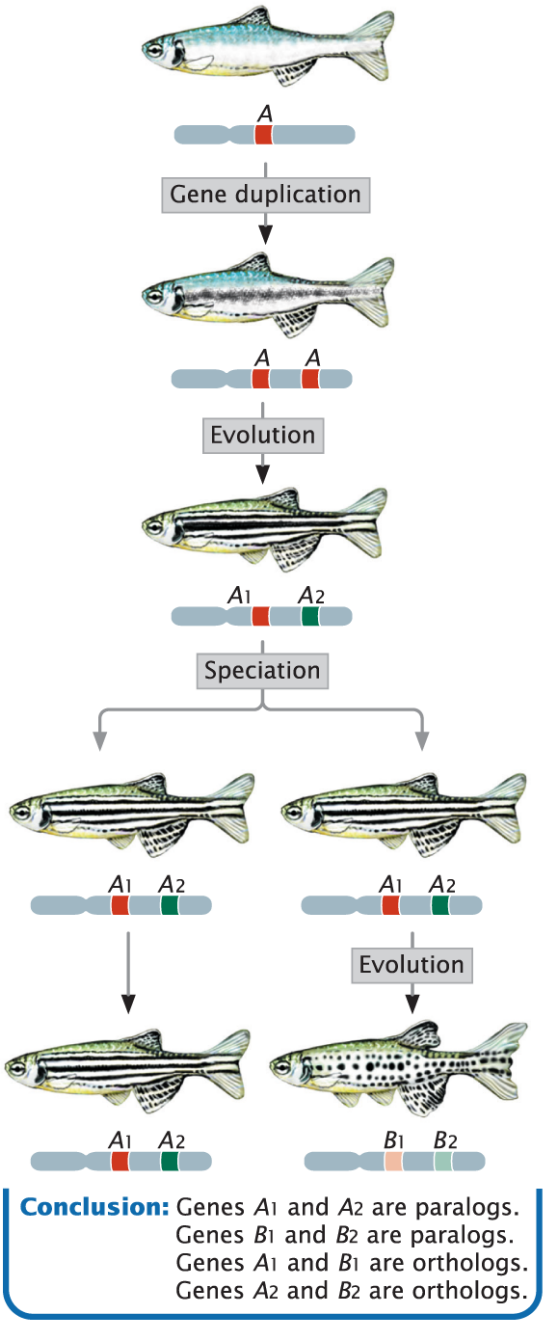
Predicting Function from Sequence

The nucleotide sequence of a gene can be used to predict the amino acid sequence of the protein it encodes. The protein can then be synthesized or isolated and its properties studied to determine its function. However, this biochemical approach to understanding gene function is both time-consuming and expensive. A major goal of functional genomics has been to develop computational methods that allow gene function to be identified from DNA sequence alone, bypassing the laborious process of isolating and characterizing individual proteins.

HOMOLOGY SEARCHES

One computational method (often the first employed) for determining gene function is to conduct a homology search, which relies on comparisons of DNA and protein sequences from the same species and from different species. Genes that are evolutionarily related, referred to as **homologous genes**, are likely to have similar sequences. Homologous genes found in different species that evolved from the same gene in a common ancestor are called **orthologs** (**Figure 20.9**). For example, both mouse and human genomes contain a gene that encodes the alpha subunit of hemoglobin; the mouse and human alpha-hemoglobin genes are said to be orthologs, because both genes evolved from an alpha-hemoglobin gene in a common mammalian ancestor. Homologous

genes in the same species (arising by duplication of a single gene in the evolutionary past) are called **paralogs** (see Figure 20.9). Within the human genome is a gene that encodes the alpha subunit of hemoglobin and another, homologous gene that encodes the beta subunit of hemoglobin. These paralogs arose because an ancestral gene underwent duplication and the resulting two genes diverged through evolutionary time, giving rise to the alpha- and beta-subunit genes (see Figure 26.19). Homologous genes (both orthologs and paralogs) often have the same or related functions, so after a function has been assigned to a particular gene, it can provide a clue to the function of a homologous gene.



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20.9 Homologous genes are evolutionarily related. Orthologs are homologous genes found in different species; paralogs are homologous genes in the same species.

Description

The flowchart starts with a fish that has gene A in its chromosome. After gene duplication, there are 2 copies of gene A in the next fish. Evolution occurs and the names of the genes in the next fish are now A 1 and A 2. Speciation occurs, so the evolutionary tree splits into two branches and there are two fish, both with genes A 1 and A 2. One of these fish evolves and the gene names are changed to B 1 and B 2. The other fish has the same genes. Text at the bottom reads, “Conclusion: Genes A 1 and A 2 are paralogs. Genes B 1 and B 2 are paralogs. Genes A 1 and B 1 are orthologs. Genes A 2 and B 2 are orthologs.”

Databases containing genes and proteins found in a wide array of organisms are available for homology searches. Powerful computer programs, such as BLAST, have been developed for scanning these databases to look for particular sequences. Suppose a geneticist sequences a genome and locates a gene that encodes a protein of unknown function. A homology search conducted on databases containing the DNA or protein sequences of other organisms may identify one or more orthologous sequences. If a function is known for a protein encoded by one of those sequences, that could provide information about the function of the newly discovered protein. Sometimes searches do not identify homologous genes; in that case, researchers may look for parts of a gene that are similar to parts of other genes with known functions. › **TRY PROBLEM 36**

PROTEIN DOMAINS

Complex proteins often contain regions, called **protein domains**, that have specific shapes or functions. For example, certain DNA-binding proteins attach to DNA in the same way; these proteins share a domain that provides the DNA-binding function. Some DNA-

binding proteins have a domain called a zinc finger, consisting of a loop of amino acids containing a zinc ion (see Chapter 16). Each protein domain has an arrangement of amino acids common to that domain (see Figure 16.2). Protein domains have been relatively stable over evolutionary time; for example, protein domains found in humans are also found in other multicellular eukaryotes. There is probably a limited, though large, number of protein domains, which have mixed and matched through evolutionary time to yield the protein diversity seen in present-day organisms.

Many protein domains have been characterized, and their molecular functions determined. The sequence from a newly identified gene can be scanned against a database of known domains. If the gene sequence encodes one or more domains whose functions have been previously determined, the functions of those domains can provide important information about possible functions of the new gene.

CONCEPTS

The function of an unknown gene can sometimes be determined by finding genes with a similar sequence whose function is known. A gene's function may also be determined by identifying functional domains in the protein it encodes.

✔ CONCEPT CHECK 5

What is the difference between orthologs and paralogs?

a. Orthologs are homologous sequences; paralogs are analogous sequences.
b. Orthologs are more similar than paralogs.
c. Orthologs are in the same species; paralogs are in different species.

d. Orthologs are in different species; paralogs are in the same species.

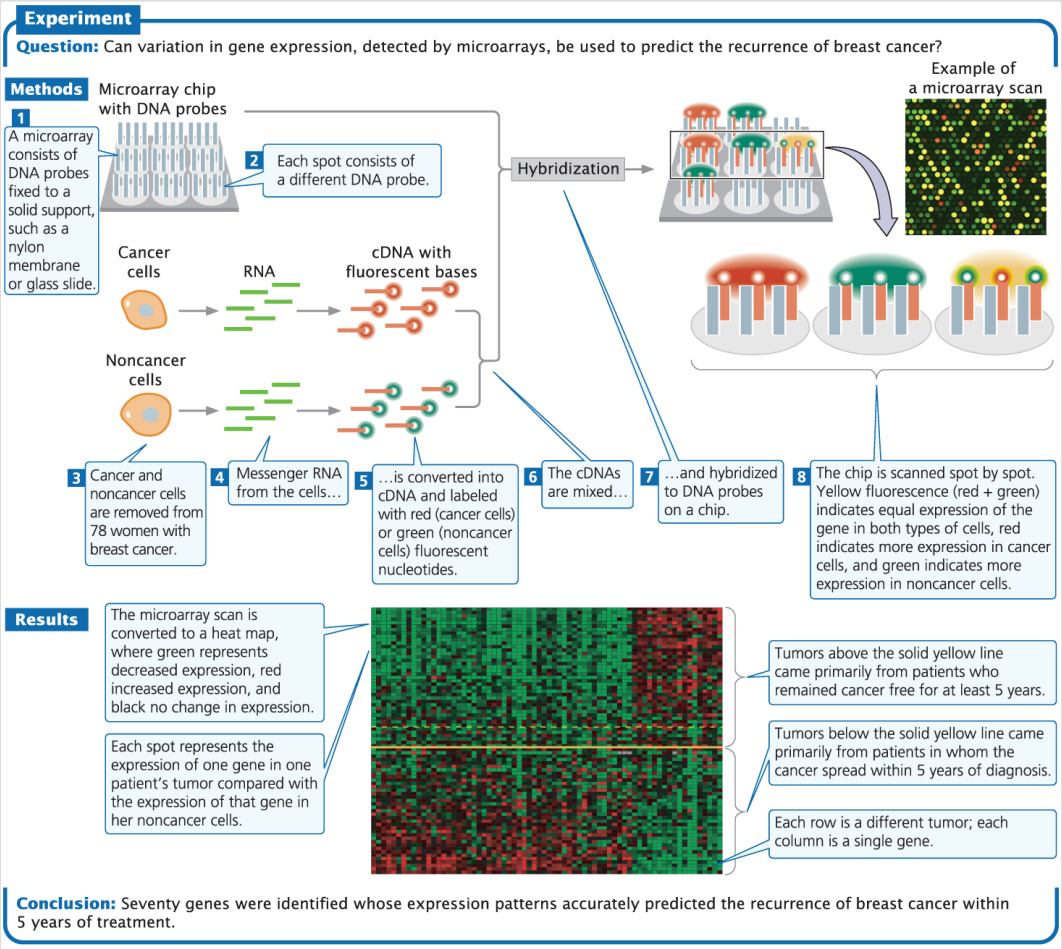
Gene Expression

Often researchers are interested not just in a DNA sequence but in how that sequence is transcribed or expressed. The expression of single genes can be studied by isolating individual RNA molecules with the use of probes or by in situ hybridization (see Section 19.4), but those methods are slow and labor-intensive when the expression profiles of many genes are required. Genomic methods provide the ability to examine the expression of thousands of genes. This approach—examining the expression of the genome—is termed **transcriptomics**. The transcriptome includes mRNAs that code for proteins, as well as other types of RNAs.

MICROARRAYS

One approach to transcriptomics uses **microarrays**, which rely on nucleic acid hybridization (see Chapter 19). In this procedure, known DNA fragments are used as probes to find complementary sequences (**Figure 20.10**). Numerous known DNA fragments are fixed to a solid support in an orderly pattern, or array, usually as a series of dots. These DNA fragments (the probes) usually correspond to known genes from a particular organism. An array containing tens of

thousands of probes can be applied to a glass slide or silicon chip just a few square centimeters in size.



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Laura J. Van'T Veer et al., Gene expression profiling predicts clinical outcome of breast cancer, *Nature* 415:532, 2002. Courtesy of EBRI. Reprinted by permission from Macmillan Publishers Ltd., ©2002, permission conveyed through Copyright Clearance Center, Inc.

20.10 Microarrays can be used to examine gene expression associated with disease progression. Each row in the microarray represents a tumor from one patient; each column is a single gene.

Description

The question in this experiment is: Can variation in gene expression, detected by microarrays, be used to predict the recurrence of breast cancer? The methods section shows microarray chips with D N A probes. Captions pointing towards these chips reads, "A microarray consists of D N A probes fixed to a solid support, such as a nylon

membrane or glass slide. Each spot consists of a different D N A probe." Then, cancer and non-cancer cells are shown. A caption reads, "Cancer and non-cancer cells are removed from 78 women with breast cancer." R N A's are obtained from both these cells, which leads to c D N A with fluorescent bases. Another caption reads, "Messenger R N A from the cells is converted into c D N A and labeled with red (cancer cells) or green (non-cancer cells) fluorescent nucleotides." The c D N A from both cells combine and are hybridized with microchips with D N A probes and a caption reads, "The c D N A's are mixed and hybridized to D N A probes on a chip." Further, it leads to a chip with various colors. At the center, three colors are shown as follows: red, green, and yellow. An example of microarray scan is shown beside. A caption reads, "The chip is scanned spot by spot. Yellow fluorescence (red plus green) indicates equal expression of the gene in both types of cells; red indicates more expression in cancer cells; and green indicates more expression in non-cancer cells." The results section shows a heat map with green and red color. Green color occupies most of the space in the map. Captions pointing to the heat map read, "The microarray scan is converted to a heat map, where green represents decreased expression, red increased expression, and black no change in expression. Each spot represents the expression of one gene in one patient's tumor compared with the expression of that gene in her non-cancer cells." The heat map is divided horizontally into two sections by a yellow line. A caption pointing towards the top section reads, "Tumors above the solid yellow line came primarily from patients who remained cancer free for at least 5 years." Two captions pointing towards the bottom section read, "Tumors below the solid yellow line came primarily from patients in whom the cancer spread within 5 years of diagnosis. Each row is a different tumor; each column is a single gene." Text at the bottom reads, "Conclusion: Seventy genes were identified whose expression patterns accurately predicted the recurrence of breast cancer within 5 years of treatment."

After the microarray has been constructed, mRNA, DNA, or cDNA isolated from experimental cells is labeled with fluorescent nucleotides and applied to the array. Any of the DNA or RNA molecules that are complementary to probes on the array will hybridize with them and emit fluorescence, which can be detected by an automated scanner.

To visualize the results, the scan of microarrays is often converted to a **heat map**. A heat map is a visual representation of a continuous variable, like the expression of a gene. The shade of the color indicates the value of the variable. For example, red might represent overexpression of the gene, green underexpression of the gene, and black average expression:

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In this way, the expression patterns of many genes can be quickly assessed (see Figure 20.10).

THE USE OF MICROARRAYS

Used with cDNA, microarrays can provide information about the expression of thousands of genes, enabling scientists to determine which genes are active in particular tissues. They can also be used to investigate how gene expression changes in the course of biological processes, such as development or disease progression. For example, breast cancer affects 1 in 8 women in the United States, and half of those women die from it. Current treatment depends on a number of factors, including a woman's age, the size of the tumor, the characteristics of the tumor cells, and whether the cancer has already spread to nearby lymph nodes. Many women whose cancer has not spread are treated by removal of the tumor and radiation

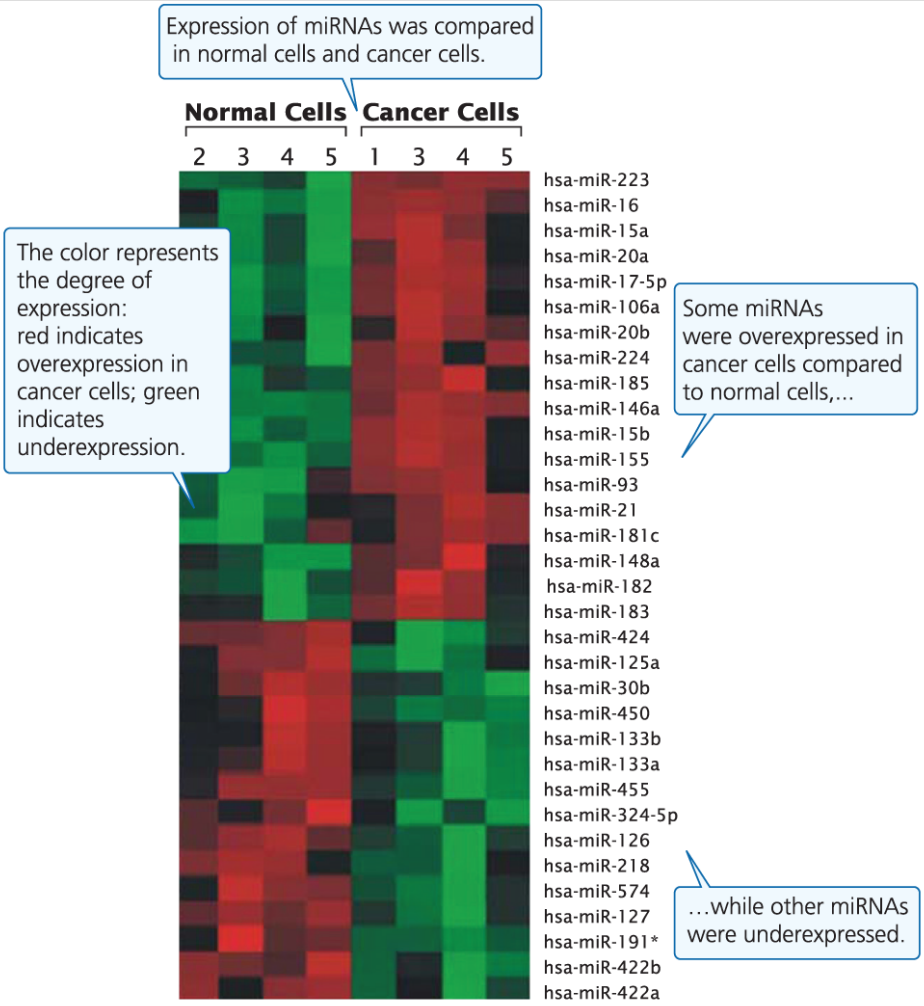
therapy, yet the cancer later reappears in some of the women thus treated. These women might benefit from more aggressive treatment when the cancer is first detected.

Using microarrays, researchers examined the expression patterns of 25,000 genes from primary tumors of 78 young women who had breast cancer (see Figure 20.10). Messenger RNA from the cancer cells and from noncancer cells was converted into cDNA and labeled with red fluorescent nucleotides and green fluorescent nucleotides, respectively. The labeled cDNAs were mixed and hybridized to a DNA chip, which contained DNA probes from numerous genes. Hybridization of the red (cancer) and green (noncancer) cDNAs was proportional to the relative amounts of mRNA in the samples. The fluorescence of each spot was assessed with microscopic scanning and appeared as a single color. Red indicated the overexpression of a gene in the cancer cells relative to its expression in the noncancer cells (more red-labeled cDNA hybridized), whereas green indicated the underexpression of a gene in the cancer cells relative to its expression in the noncancer cells (more green-labeled cDNA hybridized). Yellow indicated equal expression in both types of cells (equal hybridization of red- and green-labeled cDNAs), and no color indicated no expression in either type of cell. This information about gene expression was then converted into a heat map that displays the relative expression of genes in the tumors.

In 34 of the 78 patients, the cancer later spread to other sites; the other 44 patients remained free of breast cancer for 5 years after

their initial diagnoses. The researchers identified a subset of 70 genes whose expression patterns in the initial tumors accurately predicted whether the cancer would later spread (see Figure 20.10). This degree of prediction was much higher than that of traditional predictive measures, which are based on the size and appearance of the tumor.

Researchers have also used microarrays to examine the expression of microRNAs (miRNAs) in human cancers. Recent research indicates that miRNAs are frequently expressed abnormally in cancerous tissue and may contribute to the progression of cancer (see Section 23.2). For example, one study using microarrays found that several miRNAs were overexpressed in cancerous cervical tissue compared with normal cervical tissue, while other miRNAs were underexpressed (**Figure 20.11**). Other studies using microarrays have demonstrated that miRNA expression is associated with resistance of tumors to chemotherapy and radiation, and that miRNA expression can be used to predict the responses of some tumors to cancer treatment. Results such as these suggest that gene-expression data obtained from microarrays can be a powerful tool in cancer research and treatment. The products of the genes that show differences in expression are being examined as possible targets for drug therapy. ❯ **TRY PROBLEM 37**



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X. Wang et al., Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth, *PLoS ONE* 3(7): e2557, 2008, doi:10.1371/journal.pone.0002557.

20.11 Microarrays have been used to compare the expression of miRNAs in cancerous cervical cells with that in normal cervical cells. Shown here is a heat map showing relative expression of the miRNAs.

Description

The upper horizontal axis of the map shows labeling for normal cells divided into four columns labeled 2, 3, 4, and 5 on the left section then shows labeling for cancer cells divided into four columns labeled 1, 3, 4, and 5 on the right section. A caption reads, “Expression of m i R N A’s was compared in normal cells and cancer cells.” The right vertical axis shows various m i R N A’s as follows: h s a-m I R-223, h s a-m i R-16, h s a-

m i R-15 a, h s a-m i R-20 a, h s a-m i R-17-5p, h s a-m i R-106 a, h s a-m i R-20 b, h s a-m i R-224, h s a-m i R-185, h s a-m i R-146 a, h s a-m i R-15b, h s a-m i R-155, h s a-m i R-93, h s a-m i R-21, h s a-m i R-181 c, h s a-m i R-148 a, h s a-m i R-182, h s a-m i R-183, h s a-m i R-424, h s a-m i R-125 a, h s a-m i R-450, h s a-m i R-133 b, h s a-m i R-133 a, h s a-m i R-455, h s a-m i R-324-5 p, h s a-m i R-126, h s a-m i R-218, h s a-m i R-574, h s a-m i R-127, h s a-m i R-191, h s a-m i R-422 b, h s a-m i R-22 a. The map shows blocks of green, black, and red color. Black blocks are randomly distributed. Green blocks are at upper left and bottom right. Red blocks are at upper right and lower left. A caption reads, “Some m i R N A’s were overexpressed in cancer cells compared to normal cells, while other m i R N A’s were underexpressed.”

Microarrays that allow the detection of specific alleles, SNPs, and even particular proteins have also been created. Importantly, not all DNA molecules bind equally to microarrays, and so microarrays may sometimes overestimate or underestimate the expression of specific genes. Thus, verification of the results of microarrays through other methods is desirable. To further explore microarrays, try **Animation 20.2**.

CONCEPTS

Microarrays, consisting of DNA probes attached to a solid support, can be used to determine which RNA and DNA sequences are present in a mixture of nucleic acids. They are capable of determining which RNA molecules are being synthesized and can thus be used to examine patterns of gene expression.

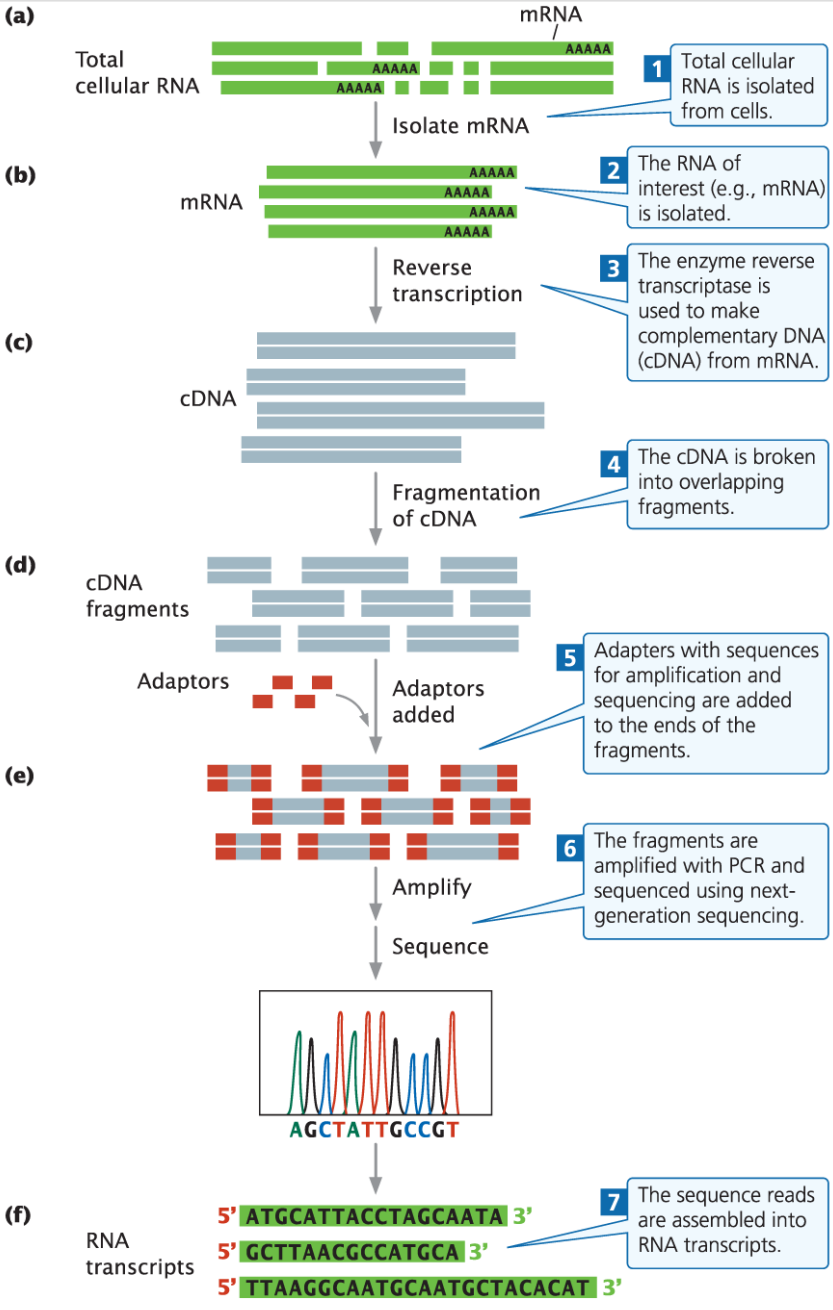
RNA SEQUENCING

Microarrays, while providing a powerful means of simultaneously examining the expression of thousands of genes, have some significant limitations. First, one must have prior knowledge of gene sequences in order to construct the array, and sometimes this information does not exist. Another limitation is that similar sequences will sometimes hybridize to the same probe on a microarray, creating cross-hybridization artifacts. A third problem is that microarrays provide a limited ability to quantify the degree of gene expression.

Another approach to the study of gene expression has been made possible by the development of rapid, low-cost next-generation sequencing methods. This approach determines the presence of RNA molecules in a cell by sequencing cDNAs copied from cellular RNA molecules. Termed **RNA sequencing**, or **RNA-seq**, this approach provides detailed information about gene expression, including the types and number of RNA molecules produced by transcription, the presence of alternatively processed RNA molecules, differential expression of the two alleles in a diploid individual, and different RNA molecules generated by bidirectional or overlapping transcription of DNA sequences.

RNA sequencing can be carried out in several ways but usually involves at least five steps (**Figure 20.12**): (1) isolation of the RNA molecules of interest from cells, (2) conversion of the RNA to complementary DNA (cDNA) sequences, (3) fragmentation and

preparation of the cDNAs for sequencing, (4) sequencing of the cDNA, and (5) assembly of the sequence reads into RNA transcripts.



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20.12 RNA sequencing can be used to determine the expression of genes.
Cellular RNA is isolated, converted to cDNA, and sequenced, providing information on the RNA transcripts present in a cell.

Description
Part “a” shows three broken strands of m R N A each having sequence A A A A A. This sequence is on the right end of the first strand and near the center in the second and third strands. It is labeled total cellular R N A. A caption reads, “Total cellular R N A is isolated from cells.” Part “b” shows only m R N A with A A A A A sequence then reverse transcription is done. A caption reads, “The R N A of interest (example, m R N A) is isolated. The enzyme reverse transcriptase is used to make complimentary D N A (c D N A) from m R N A.” Part “c” shows strands of double-stranded c D N A, which is then subjected to fragmentation. A caption reads, “The c D N A is broken into overlapping fragments.” Part “d” shows c D N A fragments. Adapters are then added at their ends. Part “e” shows adapters linked to both the left and right ends of double-stranded c D N A fragments. A caption reads, “Adapters with sequences for amplification and sequencing are added to the ends of the fragments.” The adapter linked c D N A’s are amplified and sequenced. The sequence is as follows: A G C T A T T G C C G T. A caption reads, “The fragments are amplified with P C R and sequenced using next generation sequencing.” Part “f” shows three R N A transcripts as follows: 5 prime-A T G C A T T A C C T A G C A A T A-3 prime, 5 prime-G C T T A A C G C C A T G C A-3 prime, and 5 prime-T T A A G G C A A T G C A A T G C T A C A C A T-3 prime. A caption reads, “The sequence reads are assembled into R N A transcripts.”

First, the cells are lysed and the RNA chemically extracted (Figure 20.12a). This process yields the total RNA of the cell, which includes mRNA; pre-mRNA; tRNAs; rRNA; a variety of small RNAs, such as microRNAs and siRNAs; and long noncoding RNAs. In most cells, the vast majority of RNA consists of rRNA, which often constitutes over 90% of the total cellular RNA. Usually rRNA is not the focus of study; if not removed, rRNA will be the majority of the RNA molecules that are sequenced and will reduce the detection of other RNA molecules that are of more interest. The specific RNA of interest is then separated out for sequencing (Figure 20.12b). Once isolated, the

RNA must be converted to cDNA (**Figure 20.12c**). This is done with the enzyme reverse transcriptase, which synthesizes a complementary DNA sequence from an RNA template.

After the RNA molecules are converted to cDNA, the cDNA molecules are prepared for sequencing (**Figure 20.12d**). They are broken into overlapping fragments of a size suitable for DNA sequencing, usually about 200 bp in length (sometimes the RNA is fragmented before conversion to cDNA). Adaptors—short DNA sequences necessary for amplifying and sequencing the DNA—are then added to the ends of the fragments. The cDNA is amplified using the polymerase chain reaction and sequenced using a next-generation sequencing platform (**Figure 20.12e**), such as an Illumina sequencer (see Chapter 19).

The final step is converting the readouts from the sequencing machine into full-length RNA transcripts (**Figure 20.12f**), which is accomplished by comparing overlap in the sequenced fragments. This process is often facilitated by mapping the sequence reads onto a reference genome sequence obtained by DNA sequencing.

RNA sequencing is now widely used to examine patterns of gene expression. An early application of this method was used to create a transcriptome map of yeast and revealed, surprisingly, that 75% of the nonrepetitive sequences in the yeast genome are transcribed. RNA sequencing has also been used to help identify mutations causing genetic diseases. In one study, researchers compared RNA sequences in skeleton muscle from patients with undiagnosed

skeletal disorders to those from healthy controls. The presence of abnormal RNA sequences in the patients pointed to specific mutations in the DNA that were responsible for their skeletal disorders.

Methods have been developed for isolating and sequencing RNA from single cells (termed single-cell RNA sequencing, or scRNA-seq) so that differences in gene expression among cells can be studied. For example, researchers sequenced RNA from 4645 individual cells collected from 19 patients with melanoma. The analysis revealed heterogeneity in gene expression among cells: malignant and nonmalignant cells differed in gene expression. Within the malignant cells, there were also differences, some of which were associated with resistance to anticancer drugs. Single-cell RNA sequencing is being used to characterize cellular diversity found in different tissues. For example, researchers examined gene expression patterns in over 500,000 individual cells from the mouse brain, finding several hundred types of cells based on their expression profiles. Similar studies have used scRNA-seq to define cell diversity in kidneys and the immune system. In another study, researchers studied expression patterns in individual brain cells over the lifespan of *Drosophila* and found that, as flies age, overall RNA levels of brain cells decline, as do the number of genes expressed.

CONCEPTS

RNA sequencing is used to determine the expression of genes throughout the genome. In this approach, RNA is isolated from cells and converted to cDNA, and the resulting cDNA fragments are sequenced.

Gene Expression and Reporter Sequences

Patterns of gene expression can also be determined visually by using a reporter sequence. In this approach, genomic fragments are first cloned in BACs or other vectors that are capable of holding the coding region of a gene plus its regulatory sequences. The coding region of a gene whose expression is to be studied is then replaced with a reporter sequence, which encodes an easily observed product. For example, a commonly used reporter sequence encodes a green fluorescent protein (GFP) from jellyfish. The cloned fragment is then inserted into an embryo, creating a transgenic organism. The regulatory sequences of the cloned gene ensure that it is expressed at the appropriate time and in the appropriate tissue within the transgenic organism, but the product of its expression is a green fluorescent pigment, which is easily observed (**Figure 20.13**).

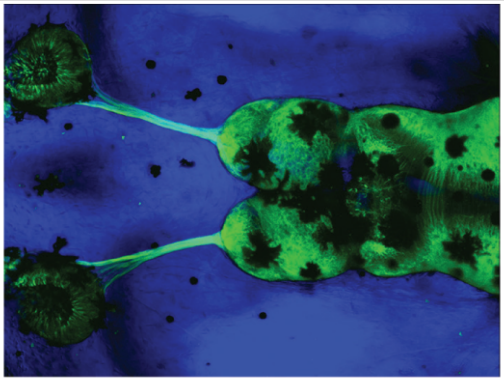


Photo by Miranda Gomperts, Courtesy Enrique Amaya, The Amaya Lab.

20.13 A reporter sequence can be used to examine expression of a gene. Expression of the neural-specific β-tubulin gene in the brain of a tadpole is revealed by green fluorescent protein.

This technique is being used to study the expression patterns of genes that affect brain function. In the Gene Expression Nervous System Atlas (GENSAT) project, scientists are systematically replacing the coding regions of hundreds of genes with the GFP reporter sequence and observing their patterns of expression in transgenic mice. The goal is to produce a comprehensive atlas of gene expression in the mouse brain. This project has already shed light on where several genes that play roles in inherited neurological disorders are expressed in the brain. Another functional genomics project, the Allen Brain Atlas, has compiled information on the expression patterns of 20,000 genes in the mouse brain.

Genome-Wide Mutagenesis

As discussed in Chapter 18, one of the best methods for determining the function of a gene is to examine the phenotypes of individual organisms that possess a mutation in the gene. Traditionally, genes encoding naturally occurring variations in a phenotype were mapped, the causative genes were isolated, and their products were studied. But this procedure was limited by the number of naturally occurring mutations and the difficulty of mapping genes with a limited number of chromosomal markers. The number of naturally occurring mutations can be increased by exposing organisms to mutagens, and the accuracy of mapping is increased dramatically by the availability of mapped molecular markers, such as SNPs. These two methods—random inducement of mutations on a genome-wide basis and mapping with molecular markers—are coupled and automated in a **mutagenesis screen**.

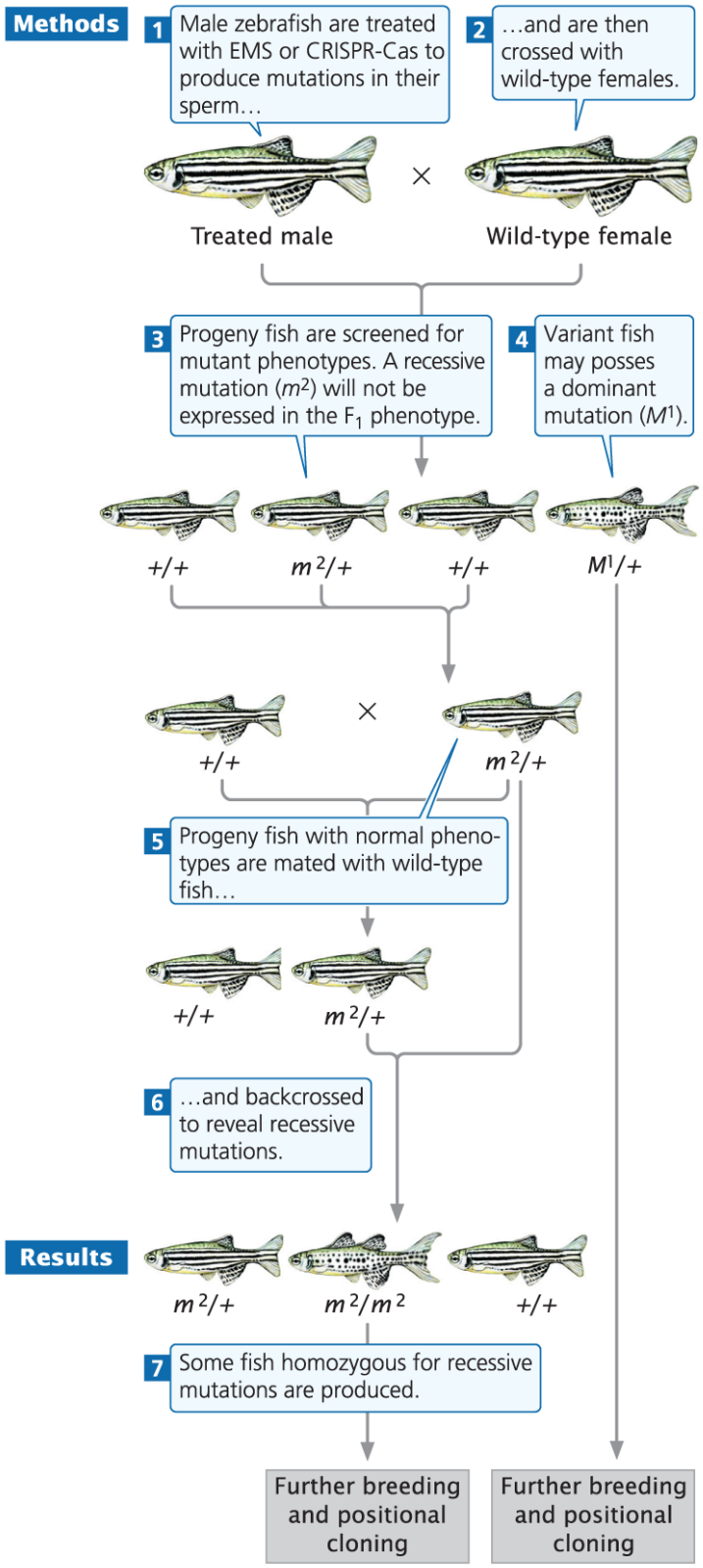
Genome-wide mutagenesis screens can be used to search for all genes affecting a particular function or trait. For example, mutagenesis screens of mice are being used to identify genes that play roles in cardiovascular function. When such genes are located in mice, homology searches are carried out to determine whether similar genes exist in humans. Those genes can then be studied to better understand cardiac disease in humans.

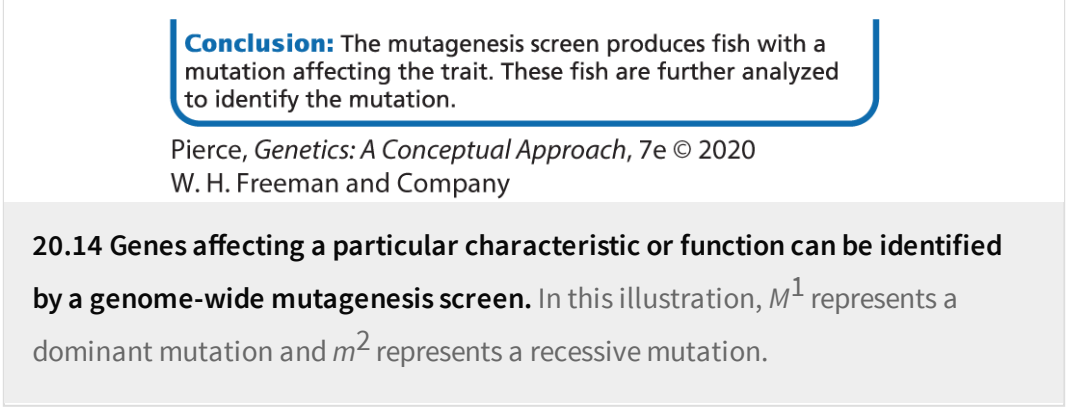
To conduct a mutagenesis screen, random mutations are induced in a population of organisms, creating new phenotypes. Mutations can be induced by exposing the organisms to radiation, chemical mutagens, or transposable elements (DNA sequences that insert

randomly into the DNA) (see Chapter 18). CRISPR-Cas (see Chapter 19) is now being widely used for mutagenesis screens. The procedure for a typical mutagenesis screen is illustrated in **Figure 20.14**. Here, male zebrafish are treated with ethylmethylsulfonate (EMS), a chemical that induces germ-line mutations in their sperm. The treated males are then mated with wild-type female fish. A few of the offspring will be heterozygous for mutations induced by EMS. The offspring are screened for any mutant phenotypes that might be the products of dominant mutations expressed in these heterozygous fish. Recessive mutations are not expressed in the F_1 progeny but can be revealed with further breeding. The fish with variant phenotypes undergo further breeding experiments to verify that each variant phenotype is, in fact, due to a single-gene mutation. The gene in which the mutation occurs is not known but can be located with positional cloning (see Chapter 19).

Experiment

Question: How can genes encoding a particular trait or function be identified?





20.14 Genes affecting a particular characteristic or function can be identified by a genome-wide mutagenesis screen. In this illustration, M^1 represents a dominant mutation and m^2 represents a recessive mutation.

Description

The question in this experiment is: How can genes encoding a particular trait or function be identified? The methods section shows a cross between a treated male fish and a wild-type female fish. A caption reads, “Male zebrafish are treated with E M S or C R I S P R-Cas to produce mutations in their sperm and are then crossed with wild-type females.” The cross leads to four fish progenies with genotype as follows: positive over positive, small M squared over positive, positive over positive, big M superscript 1 over positive. A caption pointing towards small M squared over positive fish reads, “Progeny fish are screened for mutant phenotypes. A recessive mutation (small M squared) will not be expressed in the F1 phenotype.” A caption pointing towards big M superscript 1 over positive fish reads, “Variant fish may possess a dominant mutation (M 1).” Then a cross is shown between positive over positive and small M squared over positive fish. It leads two same fishes. A caption reads, “Progeny fish with normal phenotypes are mated with wild-type fish and backcrossed to reveal recessive mutations.” Results section shows three fishes of the genotype: small M squared over positive, small M squared over small M squared, and positive over positive. Small M squared over small M squared is then subjected to further breeding and positional cloning. The fish with genotype big M superscript 1 over positive from the first cross is also subjected to further breeding and positional cloning. Text at the bottom reads, “Conclusion: The mutagenesis screen produces fish with a mutation affecting the trait. These fish are further analyzed to identify the mutation.”

Mutagenesis screens have been used to study genes that control vertebrate development. A team of developmental geneticists have

produced thousands of mutations in the zebrafish that affect development and are systematically locating and characterizing the loci where these mutations occur. Zebrafish are ideal genetic models for this type of study because they reproduce quickly, are easily reared in the laboratory, and have transparent embryos in which developmental deformities are easy to spot. This research has already identified a number of genes that are important in embryonic development, many of which have counterparts in humans.

CONCEPTS

Genome-wide mutagenesis screening coupled with positional cloning can be used to identify genes that affect a specific characteristic or function.

✔ CONCEPT CHECK 6

Which is the correct order of steps in a mutagenesis screen?

a. Positional cloning, mutagenesis, identification of mutants, verification of genetic basis.
b. Mutagenesis, positional cloning, identification of mutants, verification of genetic basis.
c. Mutagenesis, identification of mutants, verification of genetic basis, positional cloning.
d. Identification of mutants, positional cloning, mutagenesis, verification of genetic basis.

20.3 Comparative Genomics Studies How Genomes Evolve

Genome-sequencing projects provide detailed information about gene content and organization in different species and even in different members of the same species, allowing inferences about how genes function and how genomes evolve. They also provide important information about evolutionary relationships among organisms and about factors that influence the speed and direction of evolution. **Comparative genomics** is the field of genomics that studies similarities and differences in gene content, function, and organization among genomes of different organisms.

Prokaryotic Genomes

Thousands of prokaryotic genomes have now been sequenced. Most prokaryotic genomes consist of a single circular chromosome. However, there are exceptions, such as *Vibrio cholerae*, the bacterium that causes cholera, which has two circular chromosomes, and *Borrelia burgdorferi*, which has one large linear chromosome and 21 smaller chromosomes.

GENOME SIZE AND NUMBER OF GENES

The total amount of DNA in prokaryotic genomes ranges from 490,885 bp in *Nanoarchaeum equitans*, an archaean that lives entirely within another archaean, to 9,105,828 bp in *Bradyrhizobium japonicum*, a soil bacterium (Table 20.3). Although this range in genome size might seem extensive, it is much less than the enormous range of genome sizes seen in eukaryotes, which can vary from a few million base pairs to hundreds of billions of base pairs. *Escherichia coli*, the bacterium most widely used for genetic studies, has a fairly typical genome size for a prokaryote at 4.6 million base pairs. Archaea and bacteria are similar in their ranges of genome size. Surprisingly, genome size also varies extensively within some species; for example, different strains of *E. coli* vary in genome size by more than 1 million base pairs.

TABLE 20.3 Characteristics of representative prokaryotic genomes that have been completely sequenced

Species	Size (Millions of Base Pairs)	Number of Predicted Genes
Archaea		
Archaeoglobus fulgidus	2.18	2407
Methanobacterium thermoautotrophicum	1.75	1869

Nanoarchaeum equitans	0.49	536
Bacteria		
Bacillus subtilis	4.21	4100
Bradyrhizobium japonicum	9.11	8317
Escherichia coli	4.64	4289
Haemophilus influenza	1.83	1709
Mycobacterium tuberculosis	4.41	3918
Mycoplasma genitalium	0.58	480
Staphylococcus aureus	2.88	2697
Vibrio cholera	4.03	3828

Data from the Genome Atlas of the Center for Biological Sequence Analysis.

Among prokaryotes, the number of genes typically varies from 1000 to 2000, but some species have as many as 8300, and others as few as

480. The number of genes in some species of prokaryotes may be considerably higher if those that encode small RNA molecules are included. Interestingly, the density of genes is rather constant across all species, with an average of about one gene per 1000 bp. Thus, prokaryotes with larger genomes have more genes in contrast to eukaryotes, in which there is little association between genome size and number of genes, as we'll see shortly. The evolutionary factors that determine the sizes of prokaryotic genomes (as well as eukaryotic genomes) are still largely unknown. However, the time required for cell division is frequently longer in organisms with larger genomes because of the time required to replicate the DNA before division. Thus, selection may favor smaller genomes in organisms that occupy environments where rapid reproduction is advantageous.

Prokaryotes with the smallest genomes tend to be species that occupy restricted habitats, such as bacteria that live inside other organisms. The constant environment and the metabolic functions supplied by the host organism may allow these bacteria to survive with fewer genes. Bacteria with large genomes tend to occupy highly complex and variable environments, such as the soil or root nodules of plants. In these environments, genes that are needed only occasionally may be useful. In complex environments where resources are abundant, there may be little need for rapid division (which favors smaller genomes).

An example of a large-genome bacterium occupying a complex environment is *Streptomyces coeliocolor*, a filamentous bacterium with a genome size of 8,677,507 bp. This species has been called the "Boy Scout" bacterium because it has a diverse set of genes and therefore follows the Scout motto: "Be prepared." In addition to the usual housekeeping genes for genetic functions such as replication, transcription, and translation, its genome contains a number of genes for the breakdown of complex carbohydrates, allowing it to consume decaying matter from plants, animals, insects, fungi, and other bacteria. It has genes for a large number of proteins that produce secondary metabolites (breakdown products) that can function as antibiotics and protect against desiccation and low temperatures. A large genome with lots of genes appears to be beneficial in the complex environment that this species inhabits.

HORIZONTAL GENE TRANSFER

Bacteria possess a number of mechanisms by which they can gain and lose DNA. DNA can be lost through simple deletion and can be gained by gene duplication and through the insertion of transposable elements. Another mechanism for gaining new genetic information is horizontal gene transfer, a process by which both closely and distantly related bacterial species periodically exchange genetic information over evolutionary time. Such exchanges may take place through the bacterial uptake of DNA from the environment (transformation), through the exchange of plasmids,

and through viral vectors (transduction). Horizontal gene transfer has been recognized for some time, but analyses of many microbial genomes now indicate that it is more extensive than was formerly recognized. The widespread occurrence of horizontal gene transfer has caused some biologists to question whether distinct species even exist in bacteria (see Section 9.3 for more discussion of this matter).

Bacteria may even transfer genetic information to human genomes. When the human genome was first sequenced, researchers reported evidence of bacterial DNA in its sequence, but that conclusion was later questioned. More recently, researchers have reexamined this issue and have found that the human genome contains as many as 145 genes from bacteria, archaea, viruses, fungi, and other organisms. The amount of bacterial DNA is much higher in some types of cancer cells, suggesting either that cancer cells are more susceptible to horizontal gene transfer or that the insertion of bacterial DNA contributes to the progression of cancer.

CONCEPTS

Comparative genomics compares the content and organization of whole genomes of different organisms. Prokaryotic genomes are small, usually ranging from 1 million to 4 million base pairs of DNA and containing 1000 to 2000 genes. Prokaryotes with the smallest genomes tend to occupy restricted habitats, whereas those with the largest genomes are usually found in complex environments. Horizontal gene transfer has played a major role in bacterial genome evolution.

✔ CONCEPT CHECK 7

What is the relation between genome size and gene number in prokaryotes?

Eukaryotic Genomes

The genomes of many eukaryotic organisms have been completely sequenced, including those of numerous fungi and protozoans, cucumbers, papayas, corn, rice, sorghum grapevines, strawberries, silkworms, a number of fruit-fly species, aphids, mosquitoes, anemones, tunicates, ctenophores, mice, rats, dogs, pigs, sheep, cows, horses, minke whales, over 200 species of birds, orangutans, gorillas, chimpanzees, humans, and many more species. Even the genomes of some extinct organisms have now been sequenced, including those of woolly mammoths and Neanderthals. It is important to note, however, that even though the genomes of these organisms have been "completely sequenced," many of the final assembled sequences contain gaps, and regions of heterochromatin may not have been sequenced at all. Thus, the size of eukaryotic genomes is often an estimate, and the number of base pairs given as the genome size for a particular species may vary. Predicting the number of genes that are present in a eukaryotic genome is also difficult, and estimates may vary, depending on the assumptions made and the particular gene-finding software used.

GENOME SIZE AND NUMBER OF GENES

The genomes of eukaryotic organisms (**Table 20.4**) are larger than those of prokaryotes, and in general, multicellular eukaryotes have more DNA than do simple single-celled eukaryotes such as yeast (see p. 327 in Chapter 11). However, there is no close relation between genome size and complexity among the multicellular eukaryotes. For example, the mosquito *Anopheles gambiae* and the fruit fly *D. melanogaster* are both insects with similar structural complexity, yet the mosquito has 60% more DNA than does the fruit fly.

TABLE 20.4 Characteristics of representative eukaryotic genomes that have been completely sequenced

Species	Genome Size (Millions of Base Pairs)	Number of Predicted Protein-Encoding Genes
Saccharomyces cerevisiae (yeast)	12	6144
Physcomitrella patens (moss)	480	38,354
Arabidopsis thaliana (thale-cress plant)	125	25,706

Zea mays (corn)	2400	32,000
Hordeum vulgare (barley)	5100	26,159
Caenorhabditis elegans (nematode)	103	20,598
Drosophila melanogaster (fruit fly)	170	13,525
Anopheles gambiae (mosquito)	278	14,707
Danio rerio (zebrafish)	1465	22,409
Takifugu rubripes (tiger pufferfish)	329	22,089
Xenopus tropicalis (clawed frog)	1510	18,429

Anolis carolinensis (anole lizard)	1780	17,792
Mus musculus (mouse)	2627	26,762
Pan troglodytes (chimpanzee)	2733	22,524
Homo sapiens (human)	3223	~20,000

Data from the Ensembl Web site.

In general, eukaryotic genomes also contain more protein-encoding genes than do prokaryotic genomes (although some large bacteria have more genes than do single-celled yeast), and the genomes of multicellular eukaryotes have more genes than do the genomes of single-celled eukaryotes (see Table 20.4). In contrast to prokaryotes, there is no correlation between genome size and number of genes in eukaryotes. Nor is the number of genes among multicellular eukaryotes obviously related to phenotypic complexity: humans have more protein-encoding genes than do invertebrates but only twice as many as fruit flies, and fewer than the plant *A. thaliana*. The nematode *C. elegans* has more genes than *D. melanogaster* but is less complex. The pufferfish has only about one-tenth of the amount of DNA present in humans and mice but about as many genes.

Eukaryotic genomes contain multiple copies of many genes, indicating that gene duplication has been an important process in genome evolution.

Most of the DNA in multicellular eukaryotes is noncoding, and many genes are interrupted by introns. In more complex eukaryotes, both the number and the length of the introns are greater.

SEGMENTAL DUPLICATIONS AND MULTIGENE FAMILIES

Many eukaryotic genomes, especially those of multicellular organisms, are filled with **segmental duplications**, duplicated regions greater than 1000 bp that are almost identical in sequence. For example, about 4% of the human genome consists of segmental duplications. In most segmental duplications, the two copies are found on the same chromosome (intrachromosomal duplications), but in others, the two copies are found on different chromosomes (interchromosomal duplications). In the human genome, the average size of segmental duplications is 15,000 bp.

Segmental duplications arise from processes that generate chromosome duplications, such as unequal crossing over (see Section 8.2). After a segmental duplication arises, it promotes further duplication by causing misalignment among the duplicated regions. Segmental duplication plays an important role in evolution by giving rise to new genes. After a segmental duplication arises, the

original copy of the segment can continue its function while the new copy undergoes mutation. These changes may eventually lead to a new function. The importance of gene duplication in genome evolution is demonstrated by the large number of multigene families that exist in many eukaryotic genomes. A **multigene family** is a group of evolutionarily related genes that arose through repeated duplication and evolution of an ancestral gene. For example, the globin gene family in humans consists of 13 genes that encode globin-like molecules, most of which produce proteins that carry oxygen. An even more spectacular example is the human olfactory multigene family, which consists of about 850 genes that encode olfactory receptor molecules used in our sense of smell.

NONCODING DNA

Most eukaryotic organisms contain vast amounts of DNA that does not encode proteins. For example, only about 1.5% of the human genome consists of DNA that directly specifies the amino acids of proteins. Some of the remaining DNA codes for RNA molecules (such as tRNAs, rRNA, and miRNAs) and some DNA sequences (such as promoters and enhancers) play a role in regulating gene expression. However, the function of many DNA sequences has long been in question. Some research has suggested that much of this DNA has no function. For example, Marcelo Nóbrega and his colleagues genetically engineered mice that were missing large chromosomal regions with no protein-encoding genes (called **gene deserts**). In one experiment, they created mice that were missing a

1,500,000-bp gene desert from mouse chromosome 3; in another, they created mice missing an 845,000 bp gene desert from chromosome 19. Remarkably, these mice appeared healthy and were indistinguishable from control mice. The researchers concluded that large regions of the mammalian genome can be deleted without major phenotypic effects and may, in fact, be superfluous.

Other research, however, has suggested that gene deserts may contain sequences that have a functional role. For example, genome-wide association studies demonstrated that DNA sequences contained within a gene desert on human chromosome 9 are associated with coronary artery disease, and subsequent studies have demonstrated the presence of 33 enhancers in this gene desert.

In 2002, the Encyclopedia of DNA Elements (ENCODE) project was undertaken to determine whether noncoding DNA has any function. Researchers cataloged all nucleotides within the genome that provide some function, including sequences that encode proteins and RNA molecules and those that serve as control sites for gene expression. This 10-year project was carried out by a team of over 400 scientists from around the world. In a series of papers published in 2012, the ENCODE project concluded that at least 80% of the human genome is involved in some type of gene function. Many of the functional sequences consisted of sites where proteins bind and influence the expression of genes. However, the ENCODE conclusion that 80% of the genome is functional has been challenged by other researchers, and some of ENCODE's findings remain controversial.

TRANSPOSABLE ELEMENTS

A substantial part of the genomes of most multicellular organisms consists of moderately and highly repetitive sequences (see Section 11.3), and the percentage of repetitive sequences is usually higher in those species with larger genomes (**Table 20.5**). Most of these repetitive sequences appear to have arisen through transposition. In the human genome, 45% of the DNA is derived from transposable elements, many of which are defective and no longer able to move. In corn, 85% of the genome is derived from transposable elements.

TABLE 20.5 Percentages of eukaryotic genomes consisting of interspersed repeats derived from transposable elements

Organism	Percentage of Genome
Arabidopsis thaliana (thale-cress plant)	10.5
Zea mays (corn)	85.0
Caenorhabditis elegans (nematode)	6.5
Drosophila melanogaster (fruit fly)	3.1
Takifugu rubripes (tiger pufferfish)	2.7

Homo sapiens (human)	44.4

PROTEIN DIVERSITY

In spite of only a modest increase in gene number, vertebrates have considerably more protein diversity than invertebrates. One way to measure protein diversity is by counting the number of protein domains, which are characteristic parts of proteins that are often associated with a function (see Section 20.2). Vertebrate genomes do not encode significantly more protein domains than do invertebrate genomes; for example, there are 1262 domains in humans, compared with 1035 in fruit flies (**Table 20.6**). However, the existing domains in humans are assembled into more combinations, leading to many more types of proteins.

TABLE 20.6 Number of estimated protein domains encoded by some eukaryotic genomes

Organism	Number of Predicted Protein Domains
Saccharomyces cerevisiae (yeast)	851
Arabidopsis thaliana (thale-cress plant)	1012
Caenorhabditis elegans	1014

(nematode)	
Drosophila melanogaster (fruit fly)	1035
Homo sapiens (human)	1262

Data from: Number of genes and protein-domain families from the International Human Genome Sequencing Consortium, Initial sequencing and analysis of the human genome, *Nature* 409:860–921, Table 23, 2001.

HOMOLOGOUS GENES

An obvious and remarkable trend seen in eukaryotic genomes is the degree of homology among genes found in even distantly related species. For example, mice and humans share about 99% of their genes. About 50% of the genes in fruit flies are homologous to genes in humans, and even in plants, about 18% of the genes are homologous to those found in humans.

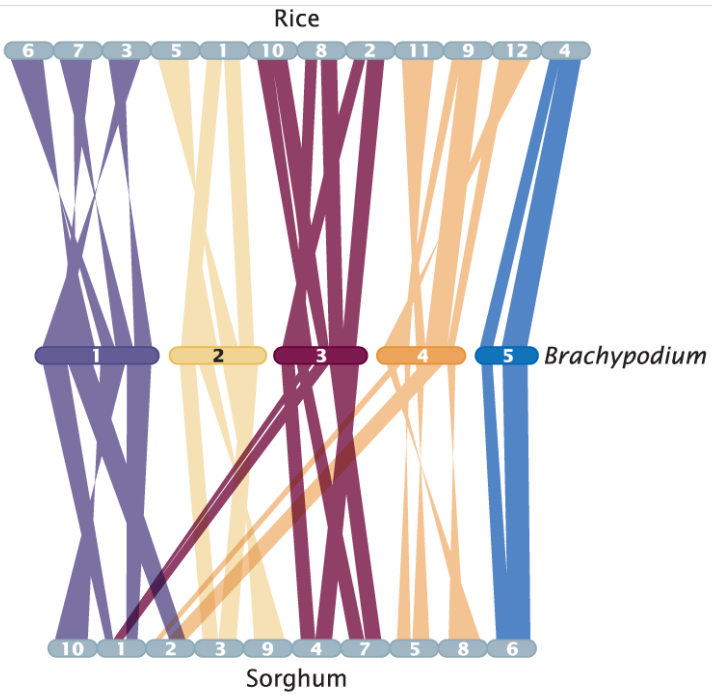
COLLINEARITY BETWEEN RELATED GENOMES

One of the features of genome evolution revealed by comparing the genome sequences of different organisms is that many genes are present in the same order in related genomes, a phenomenon that is sometimes termed *collinearity*. The reason for collinearity among genomes is that they are descended from a common ancestral genome, and evolutionary forces have maintained the same gene

order in the genomes of descendants. Genomic studies of grasses—plants in the family Poaceae—illustrate the principle of collinearity.

Grasses comprise more than 10,000 species, including economically important crops such as rice, wheat, barley, corn, millet, oats, and sorghum. Taken together, grasses make up about 60% of the world's food production. The genomes of these species vary greatly in chromosome number and size. For example, chromosome number in grasses ranges from 4 to 266; the rice genome consists of only about 460 million base pairs, whereas the wheat genome contains 17 billion base pairs. In spite of these large differences in chromosome number and genome size, the positions and order of many genes within the genomes are remarkably conserved. Over evolutionary time, regions of DNA between the genes (intergenic regions) have increased, decreased, and undergone rearrangements, whereas the genes themselves have stayed relatively constant in order and content. An example of the collinear relationships among genes in rice, sorghum, and *Brachypodium* (wild grass) is shown in **Figure 20.15**. ❯ **TRY PROBLEM 45**

THINK-PAIR-SHARE Question 6



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20.15 Collinear relationships among blocks of genes found in rice, sorghum, and *Brachypodium* (wild grass). Each colored band represents a block of genes that is collinear between chromosomes of the three species.

Description

The center of the picture shows the 5 colored bands horizontally placed that make the Brachypodium genome. Band 1 is purple, 2 is peach, 3 is black, 4 is orange, and 5 is blue. Twelve chromosomes at the top labeled from 1 to 12 randomly represent the rice genome. Ten chromosomes at the bottom labeled randomly represent the sorghum genome. Colored lines projecting from the Brachypodium chromosomes to the rice shows where the gene segments are in relation to each other. Colored bands also project downward to the sorghum chromosomes showing that many of the genes here are also present but rearranged.

CONCEPTS

Genome size varies greatly among eukaryotic species. For multicellular eukaryotic organisms, there is no clear relation between organismal complexity and amount of DNA or number of genes. A substantial part of the genome in eukaryotic organisms consists of repetitive DNA, much of which is derived from transposable elements. Many eukaryotic genomes share homologous genes, and genes are often in the same order in the genomes of related organisms.

✔ CONCEPT CHECK 8

Segmental duplications play an important role in evolution by

a. giving rise to new genes and multigene families.
b. keeping the number of genes in a genome constant.
c. eliminating repetitive sequences produced by transposition.
d. controlling the base content of the genome.

The Human Genome

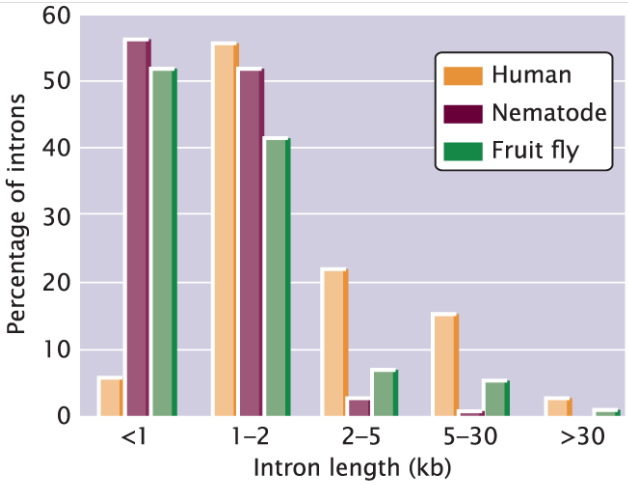
The human genome, which is fairly typical of mammalian genomes, has been extensively studied and analyzed because of its importance to human health and evolution. It is 3.2 billion base pairs in length, but only about 1.5% of it encodes proteins. Active genes are often separated by vast regions of noncoding DNA, much of which consists of repeated sequences derived from transposable elements.

The average gene in the human genome is approximately 27,000 bp in length, with about 9 exons (Table 20.7). (One exceptional gene has 234 exons.) The introns of human genes are much longer, and there are more of them than in other genomes (Figure 20.16). The

human genome does not encode substantially more protein domains (see Table 20.4), but its domains are combined in more ways to produce a relatively diverse proteome. Gene functions encoded by the human genome are presented in **Figure 20.17**. A single gene often encodes multiple proteins through alternative splicing; each gene encodes, on average, two or three different mRNAs.

TABLE 20.7 Average characteristics of genes in the human genome

Characteristic	Average
Number of exons	8.8
Size of internal exon	145 bp
Size of intron	3365 bp
Size of 5′ untranslated region	300 bp
Size of 3′ untranslated region	770 bp
Size of coding region	1340 bp
Total length of gene	27,000 bp



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20.16 The introns of genes in humans are generally longer than the introns of genes in nematodes and fruit flies.

Description

The horizontal-axis shows intron length in kilo base pairs labeled as less than 1, 1 to 2, 2 to 5, 5 to 30, and greater than 30. The vertical-axis shows percentage of introns, ranging from 0 to 60 percent with intervals of 10. The graph shows data for nematode, human, and fruit fly. Approximate data from the graph are as follows:

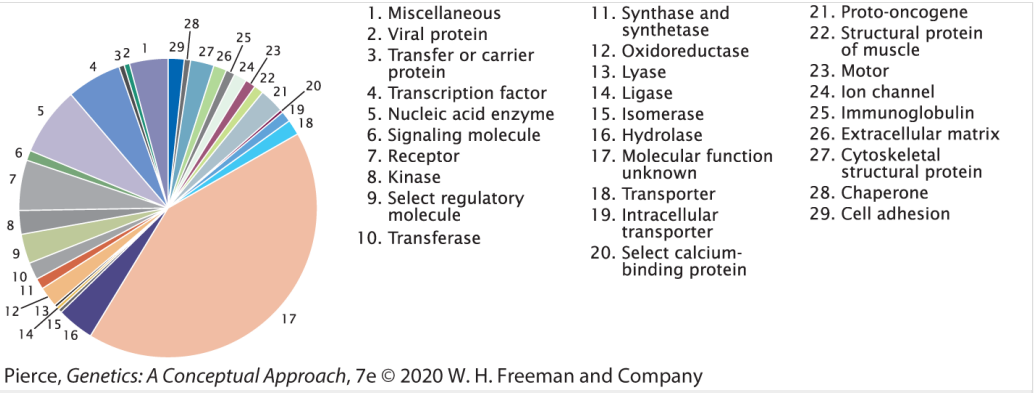
Less than 1: Human, 5; Nematode, 55; and fruit fly, 52.

1 to 2: Human, 55; Nematode, 52; and fruit fly, 42.

2 to 5: Human, 22; Nematode, 3; and fruit fly, 7.

5 to 30: Human, 15; Nematode, 1; and fruit fly, 6.

Greater than 30: Human, 5; Nematode, 0; and fruit fly, 2.



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20.17 Functions for many human genes have yet to be determined. Proportion of the circle occupied by each color represents the proportion of genes affecting various known and unknown functions.

Description

The largest area in the pie chart is occupied by molecular function unknown. The rest in approximate descending order are as follows: Nucleic acid enzyme, transcription factor, receptor, miscellaneous, hydrolase, select regulatory molecule, oxidoreductase, cytoskeletal structural protein, proto-oncogene, kinase, transferase, cell adhesion, synthase and synthetase, signaling molecule, extracellular matrix, ion channel, motor, structural protein of muscle, transporter, intracellular transporter, immunoglobulin, transfer or carrier protein, viral protein, chaperone, select calciumbinding protein, ilsomerase, ligase, and lyase.

Gene density varies among human chromosomes; chromosomes 17, 19, and 22 have the highest densities, and chromosomes X, Y, 4, 13, and 18 have the lowest. Some proteins encoded by the human genome that are not found in other animals include those affecting immune function; neural development, structure, and function; intercellular and intracellular signaling pathways in development; hemostasis; and apoptosis.

Transposable elements are much more common in the human genome than in nematode, plant, and fruit-fly genomes (see Table 20.5). The human genome contains a variety of types of transposable elements, including LINEs, SINEs, retrotransposons, and DNA transposons (see Section 18.4). Most appear to be evolutionarily old and are defective, containing mutations and deletions that make them no longer capable of transposition.

20.4 Proteomics Analyzes the Complete Set of Proteins Found in a Cell

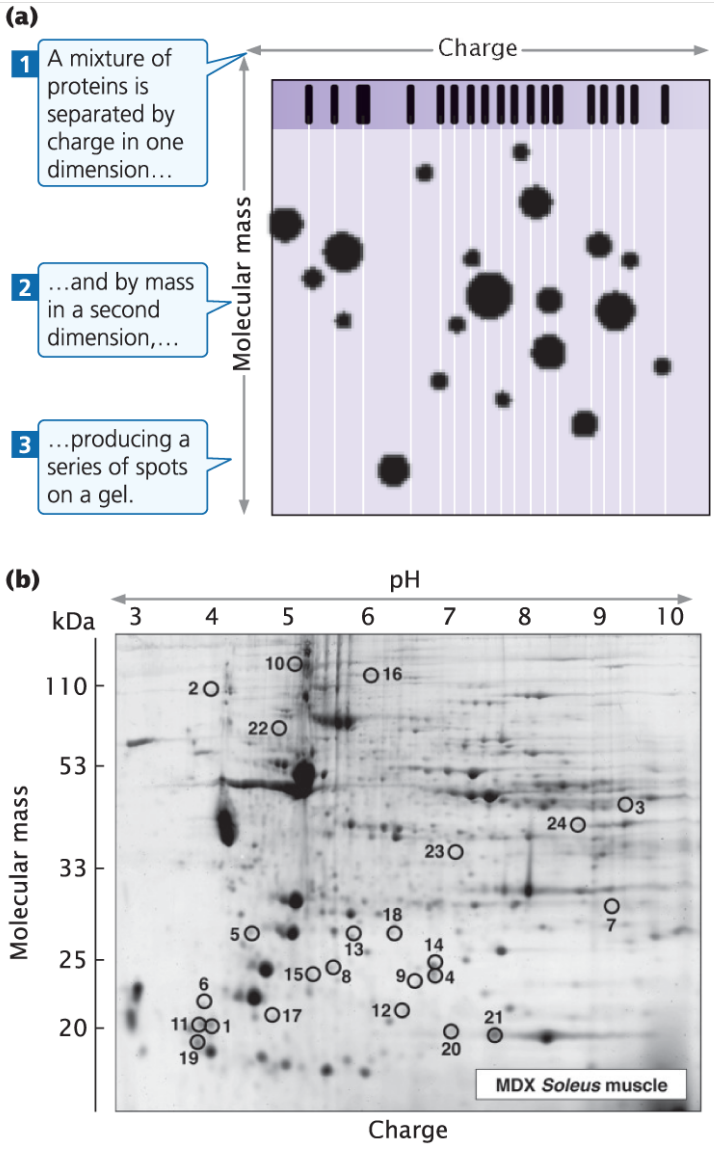
DNA sequence data offer tremendous insight into the biology of an organism, but they are not the whole story. Many genes encode proteins, and proteins carry out the vast majority of the biochemical reactions that shape the phenotype of an organism. Although proteins are encoded by DNA sequences, many proteins undergo modifications after translation, and in more complex eukaryotes, there are many more proteins than genes. Thus, in recent years, molecular biologists have turned their attention to analysis of the protein content of cells. Their ultimate goal is to determine the proteome: the complete set of proteins found in a given cell. The study of the proteome is termed **proteomics**.

Proteins make up about 50% of the dry mass of a cell; a typical cell may contain over a billion individual protein molecules. Different proteins in a cell may be present as only a few copies or over a million copies. Elucidating the complex and changing nature of the proteome is a major technical challenge. Efforts to identify and characterize all proteins in the human body has been called the **Human Proteome Project**. The project is cataloging the proteins present in each cell type, the location of each protein within the cell, and the other proteins with which each interacts. This information

will be of immense benefit in identifying drug targets, understanding the biological basis of disease, and understanding the molecular basis of many biological processes. In the future, proteomics may be coupled with genome editing, in which the findings of proteomics are tested using CRISPR-Cas (see Chapter 19) to delete or modify protein-encoding genes in specific ways, followed by additional proteomics methods to measure the effect of the gene alterations on the protein content of the cell.

Determination of Cellular Proteins

The basic procedure for characterizing the proteome is to first separate the proteins found in a cell and then to identify and quantify the individual proteins. One method for separating proteins is **two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)**, in which the proteins are separated in one dimension by charge, separated in a second dimension by mass, and then stained (**Figure 20.18a**). This procedure separates the different proteins into spots, with the size of each spot proportional to the amount of protein present. A typical 2D-PAGE gel may contain several hundred to several thousand spots (**Figure 20.18b**).



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Part b: S. Carberry et al., Comparative proteomic profiling of soleus, extensor digitorum longus, flexor digitorum brevis and interosseus muscles from the mdx mouse model of Duchenne muscular dystrophy, *Int J Mol Med* 32(3): 544–556, Figure 4, 2013.

20.18 Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) can be used to separate cellular proteins.

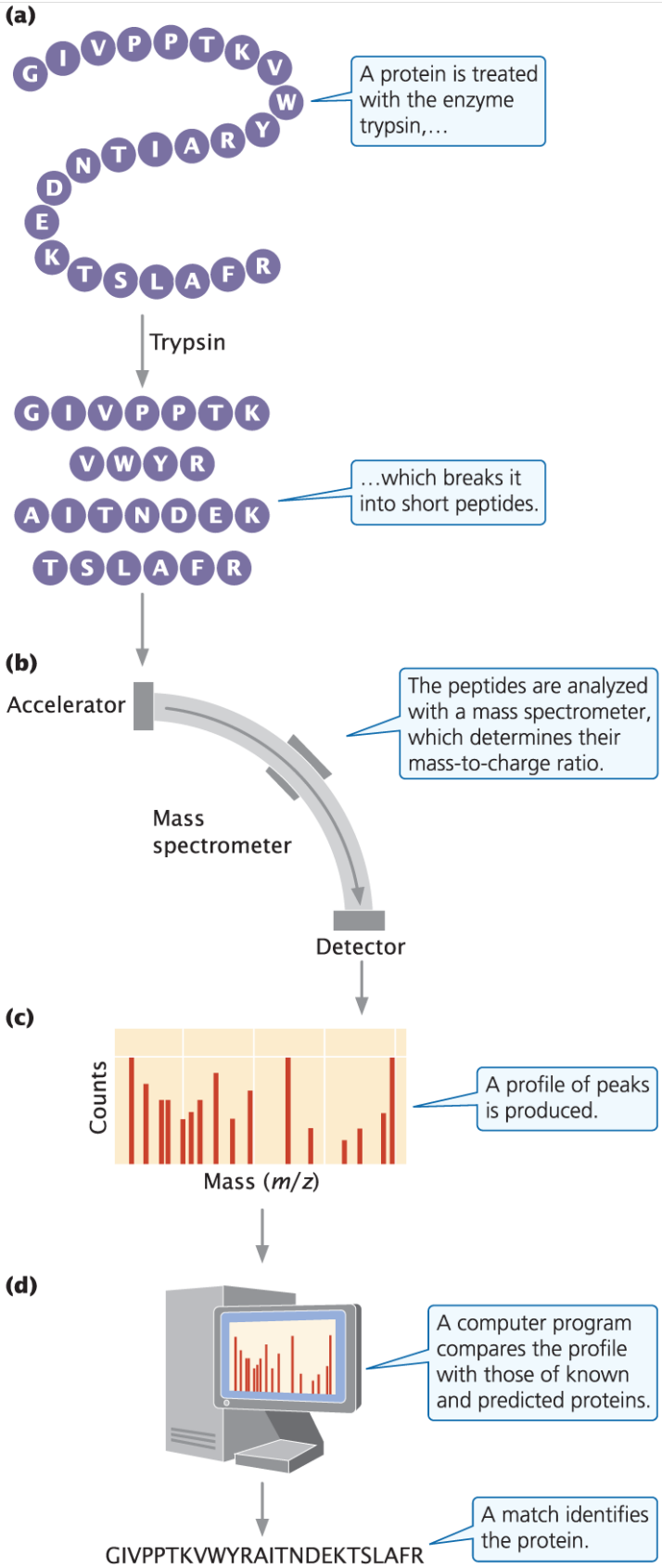
Description

Part “a” shows a drawing of a 2-D gel electrophoretic chamber with spots on it. Top of the chamber has the labeling charge. Left side of the chamber is labeled molecular mass. Spots of different sizes, representing proteins, are spread across the gel. A caption reads, “A mixture of proteins is separated by charge in one dimension and by mass in a second dimension, producing a series of spots on a gel.” Part “b” shows a photo of an actual 2-D gel electrophoresis. The top of the gel shows p H from 3 to 10. The left side represents molecular mass from 20 to 110 kilo Daltons with irregular intervals. The bottom of the gel is labeled charge. The photo has many of the protein spots labeled from 1 to 24.

Because 2D-PAGE does not detect some proteins in low abundance and is difficult to automate, researchers have turned to liquid chromatography for separating proteins. In liquid chromatography, a mixture of molecules is dissolved in a liquid and passed through a column packed with solid particles. Different affinities for the liquid and solid phases cause some components of the mixture to travel through the column more slowly than others, resulting in separation of the components of the mixture.

The traditional method for identifying a protein is to remove its amino acids one at a time and determine the identity of each one. This method is far too slow and labor-intensive for analyzing the thousands of proteins present in a typical cell. Today, researchers use **mass spectrometry**, which is a method for precisely determining the molecular mass of a molecule. In mass spectrometry, a molecule is ionized, and its migration rate in an electrical field is determined. Because small molecules migrate more rapidly than larger molecules, the migration rate can accurately determine the mass of the molecule.

To analyze a protein with mass spectrometry, researchers first digest it with the enzyme trypsin, which cleaves the protein into smaller peptide fragments, each containing several amino acids (**Figure 20.19a**). Mass spectrometry is then used to separate the peptides on the basis of their mass-to-charge ratio (**Figure 20.19b**). This separation produces a profile of peaks, in which each peak corresponds to the mass-to-charge ratio of one peptide (**Figure 20.19c**). A computer program then searches through a database of proteins to find a match between the profile generated and the profile of a known protein (**Figure 20.19d**), allowing the protein in the sample to be identified. Using bioinformatics, the computer creates "virtual digests" and predicts the profiles of all the proteins found in a genome, given the DNA sequences of the protein-encoding genes.



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20.19 Mass spectrometry is used to identify proteins.

Description
Part “a” shows a protein with the following sequence: G I V P P T K V W Y R I T N D E K T S L A F R. A caption reads, “A protein is treated with the enzyme trypsin, which breaks it into short peptides.” This protein is treated with trypsin and all the amino acids separate. It leads to part “b” which shows a mass spectrometer as a curved-tube. It has an accelerator at one end and detector at the other. A caption reads, “The peptides are analyzed with a mass spectrometer, which determines their mass-to-charge ratio.” It leads to part “c”, which shows a graph in which counts are plotted against mass in m over z. The graph shows several vertical bars. A caption reads, “A profile of peaks is produced.” It leads to part “d”, which shows the graph on a computer. A caption reads, “A computer program compares the profile with those of known and predicted proteins.” The computer then determines the sequence as G I V P P T K V W Y R I T N D E K T S L A F R. A caption reads, “A match identifies the protein.”

Mass spectrometric methods can also be used to measure the amount of each protein identified. With recent advances, researchers can now carry out “shotgun” proteomics, which eliminates most of the initial protein-separation stage. In this procedure, a complex mixture of proteins (such as those from a tissue sample) is digested and analyzed with mass spectrometry. The computer program then sorts out the proteins present in the sample by analyzing the peptide profiles.

Mary Lipton and her colleagues used this approach to study the proteome of *Deinococcus radiodurans*, an exceptional bacterium that is able to withstand high doses of ionizing radiation that are lethal to

all other organisms. The genome of *D. radiodurans* had already been sequenced. Lipton and her colleagues extracted proteins from the bacteria, digested them with trypsin into small peptide fragments, separated the fragments with liquid chromatography, and then used mass spectrometry to determine the proteins from the peptide fragments. They were able to identify 1910 proteins, which represented more than 60% of the proteins predicted on the basis of the genome sequence.

Deciphering the proteome of even a single cell is a challenging task. Every cell contains a complete sequence of genes, but different cells express vastly different proteins. Each gene may produce a number of different proteins through alternative processing (see Chapter 14) and posttranslational protein processing (see Chapter 15). At present, proteomic analyses of the most intensively studied cells have detected a little over 10,000 different proteins, although analysis of mRNA transcripts suggests that the total number of proteins produced is considerably higher.

Affinity Capture

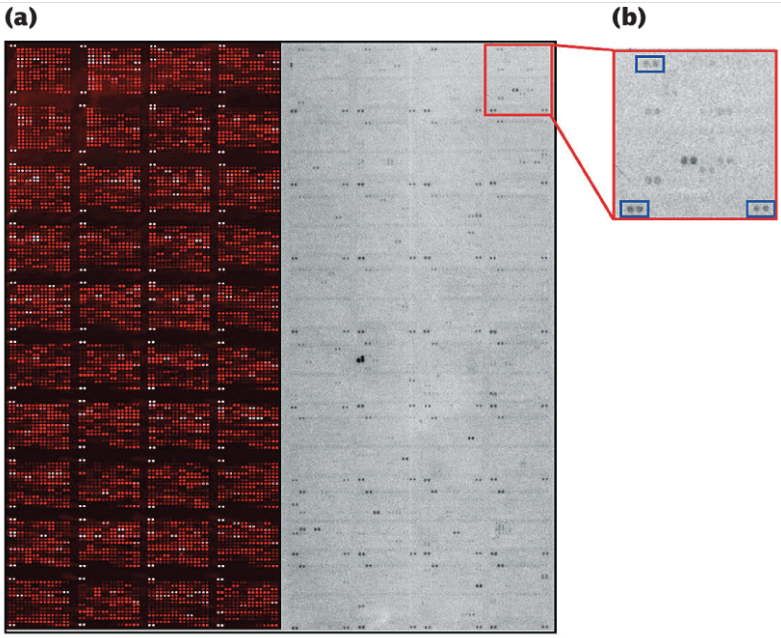
Proteomics concerns not just the identification of all proteins in a cell but also an understanding of how these proteins interact and how their expression varies with the passage of time. Researchers have developed a number of techniques for identifying proteins that interact within the cell. In **affinity capture**, an antibody (see Figure

22.22) to a specific protein is used to capture one protein from a complex mixture of proteins. The protein captured will "pull down" with it any proteins with which it physically interacts. The pulled-down mixture of proteins can then be analyzed by mass spectrometry to identify the proteins.

Modifications of affinity capture and other techniques can be used to determine the complete set of protein interactions in a cell, termed the **interactome**. In a project called BioPlex 2.0, affinity capture has been coupled with mass spectrometry to produce a collection of over 56,000 interactions from almost 11,000 different proteins.

Protein Microarrays

Protein–protein interactions can also be analyzed with **protein microarrays** (**Figure 20.20**), which are similar to the microarrays used for examining gene expression. With this technique, a large number of different proteins are applied to a solid support in an orderly array of spots, with each spot containing a different protein. In one application, each spot contains an antibody for a different protein, labeled with a tag that fluoresces when bound. An extract of tissue is applied to the microarray. A spot of fluorescence appears when a protein in the extract binds to an antibody, indicating the presence of that particular protein in the tissue.



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From D. Hall, J. Ptacek, and M. Snyder, Protein microarray technology, *Mechanisms of Ageing and Development* 128: 161–167, 2007. Republished with permission of Elsevier, permission conveyed through Copyright Clearance Center, Inc.

20.20 Protein microarrays can be used to examine interactions among proteins. (a) A microarray containing 4400 proteins found in yeast. (b) The array was probed with an enzyme that phosphorylates proteins to determine which proteins serve as substrate for the enzyme. Dark spots represent proteins that were phosphorylated by the enzyme. Proteins that phosphorylate themselves (autophosphorylate) are included in each block of the microarray (shown in blue boxes) to serve as reference points.

Structural Proteomics

The high-resolution three-dimensional structure of a protein provides a great deal of useful information. It is often a source of insight into the function of an unknown protein; it may also suggest

the locations of active sites and provide information about other molecules that interact with the protein. Furthermore, knowledge of a protein's structure often suggests targets for potential drugs that might interact with the protein. Therefore, one goal of proteomics is to determine the structure of every protein found in a cell.

Two procedures are currently used to solve the structures of complex proteins: (1) X-ray crystallography, in which crystals of the protein are bombarded with X-rays, and the diffraction patterns of the X-rays are used to determine the structure (see Section 10.2); and (2) nuclear magnetic resonance (NMR), which provides information on the positions of specific atoms within a molecule by using the magnetic properties of their nuclei.

Both X-ray crystallography and NMR require human intervention at many stages and are too slow for determining the structures of the thousands of proteins that may exist within a cell. Because the structures of hundreds of thousands of proteins are required for studies of the proteome, researchers ultimately hope to be able to predict the structure of a protein from its amino acid sequence. Such predictions are not possible at the present time, but the hope is that if the enough high-resolution structures are solved, it will be possible in the future to model a protein's structure from the amino acid sequence alone. As scientists work on automated methods that will speed the structural determination of proteins, bioinformaticians are developing better computer programs for predicting protein structure from sequence.

CONCEPTS

The proteome is the complete set of proteins found in a cell. Techniques of protein separation and mass spectrometry are used to identify the proteins present within a cell. Affinity capture and microarrays are used to determine sets of interacting proteins. Structural proteomics attempts to determine the structures of all proteins.

✔ CONCEPT CHECK 9

Why is knowledge of a protein's structure important?

Chapter 20 Review

CHAPTER SUMMARY

Section 20.1 Learning Objective: Be able to describe how whole genomes are sequenced.

- Genomics is the field of genetics that attempts to understand the content, organization, and function of the genetic information contained in whole genomes.
- Genetic maps position genes relative to other genes by determining rates of recombination and are measured in recombination frequencies. Physical maps are based on the physical distances between genes and are measured in base pairs.
- The Human Genome Project was an effort to determine the entire sequence of the human genome. The project began officially in 1990; rough drafts of the human genome sequence were completed in 2000. The final draft of the human genome sequence was completed in 2003.
- Sequencing a whole genome requires breaking it into small overlapping fragments whose DNA sequences can be determined in sequencing reactions. The individual sequences can be ordered into a whole-genome sequence with a map-based approach, in which fragments are assembled by using previously created genetic and physical maps, or with a whole-

genome shotgun approach, in which overlap between fragments is used to assemble them into a whole-genome sequence. Today, almost all genomes are sequenced using whole-genome shotgun sequencing.

- Single-nucleotide polymorphisms are single-base differences in DNA between individual organisms and are valuable as markers in linkage studies. Individual organisms may also have differences in their numbers of copies of DNA sequences, called copy-number variations.
- Bioinformatics is a synthesis of molecular biology and computer science that develops tools to store, retrieve, and analyze DNA-, RNA-, and protein-sequence data.
- Metagenomics studies the genomes of entire groups of organisms. Synthetic biology is developing techniques for creating genomes and organisms.

Section 20.2 Learning Objective: Be able to describe genomic approaches to understanding gene function.

- Homologous genes are evolutionarily related. Orthologs are homologous sequences found in different species, whereas paralogs are homologous sequences found in the same species. Gene function may be determined by looking for homologous sequences (both orthologs and paralogs) whose function has been previously determined.
- A microarray consists of known DNA fragments, fixed in an orderly pattern to a solid support, that act as probes. When a solution containing a mixture of DNA or RNA is applied to the

array, any nucleic acid that is complementary to one of the probes will bind to that probe. Microarrays can be used to monitor the expression of thousands of genes simultaneously.
- RNA sequencing analyzes the expression of genes throughout the genome. In this approach, RNA is isolated from cells and converted to cDNA, and the resulting cDNA fragments are sequenced.
- By linking a reporter sequence to the regulatory sequences of a gene, the expression pattern of the gene can be observed by looking for the product of the reporter sequence. Genes affecting a particular function can also be identified through whole-genome mutagenesis screens.

Section 20.3 Learning Objective: Be able to compare and contrast prokaryotic and eukaryotic genomes.

- Most prokaryotic species have between 1 million and 4 million base pairs of DNA and between 1000 and 2000 genes. The density of genes in prokaryotic genomes is relatively uniform, with about one gene per 1000 bp. Horizontal gene transfer (the movement of genes between different species) has been an important evolutionary process in prokaryotes.
- Eukaryotic genomes are larger and more variable in size than are prokaryotic genomes. There is no clear relation between organismal complexity and the amount of DNA or number of genes among multicellular organisms. Much of the genomes of eukaryotic organisms consist of repetitive DNA. Transposable elements are very common in most eukaryotic genomes.

Section 20.4 Learning Objective: Be able to describe how proteomics provides information about the protein content of a cell.

- Proteomics determines the protein content of a cell and the functions of those proteins. Proteins within a cell can be separated and identified with the use of mass spectrometry. Structural proteomics attempts to determine the three-dimensional shapes of proteins.

WORKED PROBLEM

Problem

A linear piece of DNA that is 30 kb long is first cut with *Bam*HI, then with *Hpa*II, and finally with both *Bam*HI and *Hpa*II together. Fragments of the following sizes are obtained from these reactions:

*Bam*HI: 20-kb, 6-kb, and 4-kb fragments

*Hpa*II: 21-kb and 9-kb fragments

*Bam*HI and *Hpa*II: 20-kb, 5-kb, 4-kb, and 1-kb fragments

Draw a restriction map of the 30-kb piece of DNA, indicating the locations of the *Bam*HI and *Hpa*II restriction sites.

» Solution Strategy

What information is required in your answer to the problem?

A map that includes the number and relative locations of restriction sites for *Bam*HI and *Hpa*II and the distances in base pairs between the sites.

What information is provided to solve the problem?

- The piece of DNA is 30 kb long.
- The sizes of the fragments produced when the DNA is cut with *Bam*HI, with *Hpa*II, and with both enzymes together.

For help with this problem, review:

Physical Maps in Section 20.1.

» Solution Steps

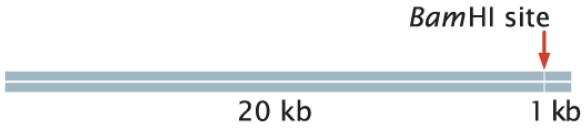
When cut by *Bam*HI alone, the linear piece of DNA is cleaved into three fragments, so there must be two *Bam*HI restriction sites. When cut with *Hpa*II alone, a clone of the same piece of DNA is cleaved into only two fragments, so there is a single *Hpa*II site.

Note: This problem can be solved correctly through a variety of approaches; this solution applies one possible approach.

Hint: For linear DNA, the number of restriction sites is one less than the number of fragments produced.

Let's begin to determine the locations of these sites by examining the *Hpa*II fragments. Notice that the 21-kb fragment produced when the DNA is cut by *Hpa*II is not present in the fragments produced when the DNA is cut by *Bam*HI and *Hpa*II together (the double digest); this result indicates that the 21-kb *Hpa*II fragment has within it a *Bam*HI site. If we examine the fragments produced by the double digest, we see that the 20-kb and 1-kb fragments sum to 21 kb; thus, a *Bam*HI site must be 20 kb from one end of the fragment and 1 kb from the other end.

Hint: Look for fragments in the double digest that sum to the length of a fragment present in the single digest.



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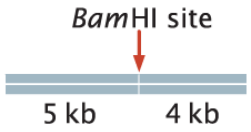
Similarly, we see that the 9-kb *Hpa*II fragment does not appear in the double digest and that the 5-kb and 4-kb fragments in the double digest add up to 9 kb; so another *Bam*HI site must be 5 kb from one end of this fragment and 4 kb from the other end.



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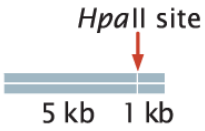
Now let's examine the fragments produced when the DNA is cut by *Bam*HI alone. The 20-kb and 4-kb fragments are also present in the double digest, so neither of these fragments contains an *Hpa*II site. The 6-kb fragment, however, is not present in the double digest, and the 5-kb and 1-kb fragments in the double digest sum to 6 kb, so this fragment contains an *Hpa*II site that is 5 kb from one end and 1 kb from the other end.



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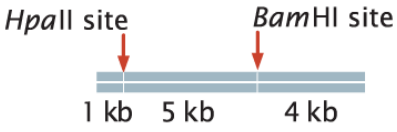
We have accounted for all the restriction sites, but we still must determine the order of the sites on the original 30-kb fragment. Notice that the 5-kb fragment must be adjacent to both the 1-kb and the 4-kb fragments, so it must be in between those two fragments.



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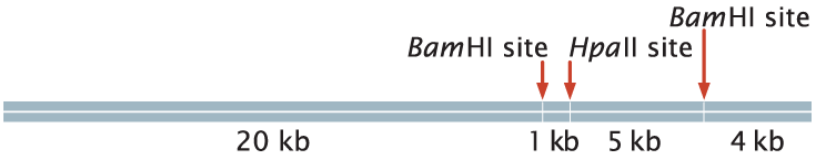
Hint: Two fragments in the double digest that were produced by cutting a fragment in the single digest must be adjacent to one another.

We have also established that the 1-kb and 20-kb fragments are adjacent; because the 5-kb fragment is on one side, the 20-kb fragment must be on the other, completing the restriction map:



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IMPORTANT TERMS

genomics
structural genomics
genetic (linkage) map
physical map
map-based sequencing
contig
whole-genome shotgun sequencing
sequencing coverage
single-nucleotide polymorphism (SNP)
haplotype
tag-SNP
genome-wide association study

bioinformatics
annotation
metagenomics
microbiome
synthetic biology
functional genomics
transcriptome
proteome
homologous gene
orthologous gene
paralogous gene
protein domain
transcriptomics
microarray
heat map
RNA sequencing
mutagenesis screen
comparative genomics
segmental duplication
multigene family
gene desert
proteomics
Human Proteome Project
two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)
mass spectrometry
affinity capture
interactome
protein microarray

ANSWERS TO CONCEPT CHECKS

1. Two limitations are accuracy and resolution.
2. b
3. It cataloged and mapped SNPs and other human genetic variants that could be used to identify common haplotypes in human populations.
4. a
5. d
6. c
7. Prokaryotic species with larger genomes generally have more genes than do species with smaller genomes, so gene density is quite constant.
8. a
9. Structure often provides important information about how a protein functions and the types of proteins with which it is likely to interact.

Chapter 20 Assessment

COMPREHENSION QUESTIONS

Section 20.1

1. What is the difference between a genetic map and a physical map? Which generally has higher resolution and accuracy, and why?
2. What is the difference between a map-based approach to sequencing a whole genome and a whole-genome shotgun approach?
3. What are some of the ethical concerns arising out of the information produced by the Human Genome Project?
4. What is a single-nucleotide polymorphism (SNP)? How are SNPs used in genomic studies?
5. What is a haplotype? How do different haplotypes arise?
6. What is linkage disequilibrium? How does it result in haplotypes?
7. How is a genome-wide association study carried out?
8. What is copy-number variation? How does it arise?
9. How are genes recognized within genome sequences?

Section 20.2

10. What are homologous sequences? What is the difference between orthologs and paralogs?

11. Describe several different methods for inferring the function of a gene by examining its DNA sequence.

12. What is a microarray? How can it be used to obtain information about gene function?

13. How are RNA molecules sequenced?

14. Explain how a reporter sequence can be used to provide information about the expression pattern of a gene.

15. Briefly outline how a mutagenesis screen is carried out.

Section 20.3

16. What is the relation between genome size and gene number in prokaryotes?

17. What is horizontal gene transfer? How might it take place between different species of bacteria?

18. Genome size varies considerably among multicellular organisms. Is this variation closely related to the number of genes and the complexity of the organism? If not, what accounts for some of this variation?

19. More than half of the genome of *A. thaliana* consists of duplicated sequences. What mechanisms are thought to have been responsible for these extensive duplications?

20. What is a segmental duplication?

21. The human genome does not encode substantially more protein domains than do invertebrate genomes, yet it encodes many more proteins. How are more proteins encoded when the number of domains does not differ substantially?

22. What is genomics, and how does structural genomics differ from functional genomics? What is comparative genomics?

Section 20.4

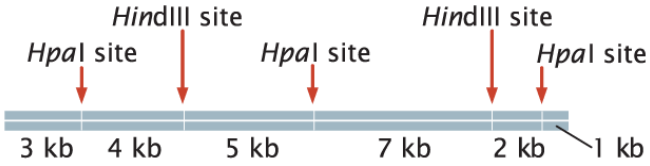
23. How does proteomics differ from genomics?

24. How is mass spectrometry used to identify proteins in a cell?

APPLICATION QUESTIONS AND PROBLEMS

Section 20.1

25. *A 22-kb piece of DNA has the following restriction sites:



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A batch of this DNA is first fully digested by *Hpa*I alone, then another batch is fully digested by *Hin*dIII alone, and finally, a third batch is fully digested by both *Hpa*I and *Hin*dIII together. The fragments resulting from each of the three digestions are placed in separate wells of an agarose gel, separated by gel electrophoresis, and stained by ethidium bromide. Draw the bands as they would appear on the gel.

26. *A linear piece of DNA that is 14 kb long is cut first by *Eco*RI alone, then by *Sma*I alone, and finally by both *Eco*RI and *Sma*I together. The following results are obtained:

Digestion by *Eco*RI alone	**Digestion by *Sma*I alone**	**Digestion by both *Eco*RI and *Sma*I**
3-kb fragment	7-kb fragment	2-kb fragment

5-kb fragment	7-kb fragment	3-kb fragment
6-kb fragment		4-kb fragment
		5-kb fragment

Draw a map of the *Eco*RI and *Sma*I restriction sites on this 14-kb piece of DNA, indicating the relative positions of the restriction sites and the distances between them.

27. *The presence (+) or absence (–) of six sequences in each of five bacterial artificial chromosome (BAC) clones (A–E) is indicated in the following table. Using these markers, put the BAC clones in their correct order, and indicate the locations of the numbered sequences within them.

	Sequences					
BAC clone	**1**	**2**	**3**	**4**	**5**	**6**
A	+	–	–	–	+	–
B	–	–	–	+	–	+
C	–	+	+	–	–	–
D	–	–	+	–	+	–
E	+	–	–	+	–	–

28. A linear piece of DNA was broken into random, overlapping fragments, and each fragment was sequenced. The sequence of each fragment is shown below.

Fragment 1: 5′–TAGTTAAAAC–3′

Fragment 2: 5′–ACCGCAATACCCTAGTTAAA–3′

Fragment 3: 5′–CCCTAGTTAAAAC–3′

Fragment 4: 5′–ACCGCAATACCCTAGTT–3′

Fragment 5: 5′–ACCGCAATACCCTAGTTAAA–3′

Fragment 6: 5′–ATTTACCGCAAT–3′

On the basis of overlap in sequence, create a contig sequence of the original piece of DNA.

29. CONNECT In Chapter 11, we discussed three different types of sequences found in eukaryotic genomes: unique sequence DNA, moderately repetitive DNA, and highly repetitive DNA. Which of these sequences do you think would be most difficult to assemble into a complete genome sequence and why?

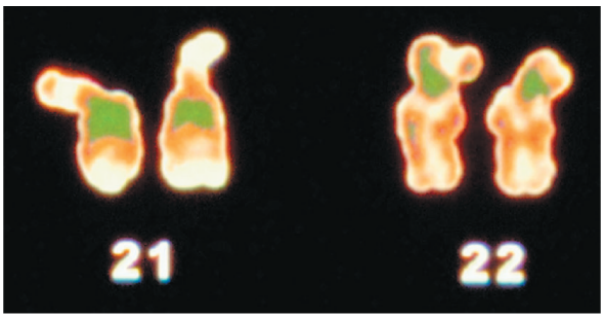
30. *How does the density of genes found on chromosome 22 compare with the density of genes found on chromosome 21, two similar-sized chromosomes? How does the number of genes on chromosome 22 compare with the number found on the Y chromosome?

    To answer these questions, go to www.ensembl.org. Under the heading *Species*, select *Human*. On the next page, click on *View Karyotype*. Pictures of the human chromosomes will appear. Click on chromosome 22 and select *Chromosome Summary*. You will be shown a picture of this chromosome and histograms of known genes (colored bars). The total numbers of coding (protein-encoding) genes, along with the chromosome length in base pairs, are given in the table at the bottom of the diagram. Write down the total length of the chromosome and the number of coding genes.

    Now go to chromosome 21 by selecting it from the *Change Chromosome* drop-down. Examine the total length and total number of protein-encoding genes

for chromosome 21. Now do the same for the Y chromosome. Calculate the gene density (number of genes/length) for chromosomes 22, 21, and Y.

a. Which chromosome has the highest density and greatest number of genes? Which has the fewest?
b. Examine in more detail the genes at the tip of the short arm of the Y chromosome by clicking on the top bar in the histogram of genes. Jump to location view. A more detailed view will be shown. What known genes are found in this region? How many protein-encoding genes are there in this region?



Leonard Lessin/Science Source.

Human chromosomes 21 and 22.

31. DATA ANALYSIS In recent years, honeybee colonies throughout North America have been decimated by colony collapse disorder (CCD), which results in the rapid deaths of worker bees. First noticed by beekeepers in 2004, the disorder has been responsible for the loss of 50% to 90% of beekeeping operations in the United States. Evidence suggests that CCD is caused by a pathogen. Diana Cox-Foster and her colleagues (D. Cox-Foster et al. 2007. *Science* 318:283–287) used a metagenomic approach to try to identify the causative agent of CCD by isolating DNA from normal honeybee hives and from hives that had experienced CCD. A number of different bacteria, fungi, and viruses were identified in the metagenomic analysis. The following table gives the percentage of CCD hives and non-CCD hives that tested positive for four potential pathogens identified in the metagenomic analysis. On the basis of these data, which potential pathogen appears most likely to be responsible

for CCD? Explain your reasoning. Do these data prove that this pathogen is the cause of CCD? Explain.

Potential pathogen	CCD hives infected (*n* = 30)	Non-CCD hives infected (*n* = 21)
Israeli acute paralysis virus	83.3%	4.8%
Kashmir bee virus	100%	76.2%
Nosema apis	90%	47.6%
Nosema cernae	100%	80.8%

32.

Give some specific examples of how genome sequencing has increased our understanding of early human evolution. (Hint: See the introduction to Chapter 10.)

33.

James Noonan and his colleagues (J. Noonan et al. 2005. *Science* 309:597–599) set out to study the genome sequence of an extinct species of cave bear. They extracted DNA from 40,000-year-old bones of a cave bear and used a metagenomic approach to isolate, identify, and sequence the cave-bear DNA. Why did they use a metagenomic approach when their objective was to sequence the genome of one species (the cave bear)?

Larry Miller/Science Source.

34. CONNECT Explain how third-generation sequencing methods (see Chapter 19) have facilitated the sequencing of whole genomes.

Section 20.2

35. Explain why genes A_2 and B_2 in **Figure 20.9** are orthologs and not paralogs.

36. *Examine **Figure 26.19**. Are the epsilon (ε) and beta (β) genes on chromosome 11 orthologs or paralogs? Explain your answer.

37. *Microarrays can be used to determine relative levels of gene expression. In one type of microarray, hybridization of red (experimental) and green (control) labeled cDNAs is proportional to the relative amounts of mRNA in the samples. Red indicates the overexpression of a gene, and green indicates the underexpression of a gene, in the experimental cells relative to the control cells; yellow indicates equal expression in experimental and control cells; and no color indicates no expression in either experimental or control cells.

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Description
Circles 2 and 24 are red. Circles 4, 17, and 22 are green. Circles 1, 13, 14, and 21 are white, and the other 16 circles are yellow.

In one experiment, mRNA from a strain of antibiotic-resistant bacteria (experimental cells) is converted into cDNA and labeled with red fluorescent nucleotides; mRNA from a nonresistant strain of the same bacteria (control cells) is converted into cDNA and labeled with green fluorescent nucleotides. The cDNAs from the resistant and nonresistant cells are mixed and hybridized to a chip containing spots of DNA from genes 1 through 25. The results are shown in the illustration above. What conclusions can you draw about which genes might be implicated in antibiotic resistance in these bacteria? How might this information be used to design new antibiotics that are less vulnerable to resistance?

38. Of the genes in the microarray shown in the lower part of **Figure 20.10**, are most overexpressed or underexpressed in tumors from patients that remained cancer free for at least five years? Explain your reasoning.

39. What does the photograph in **Figure 20.13** reveal about the expression of β-tubulin?

40. CONNECT Much research has been devoted to developing methods for detecting, sequencing, and measuring levels of RNA (see Chapters 23 and 13) within the cell. All RNA is transcribed from DNA, so what information does RNA provide that is not found in DNA sequences? Give at least three examples.

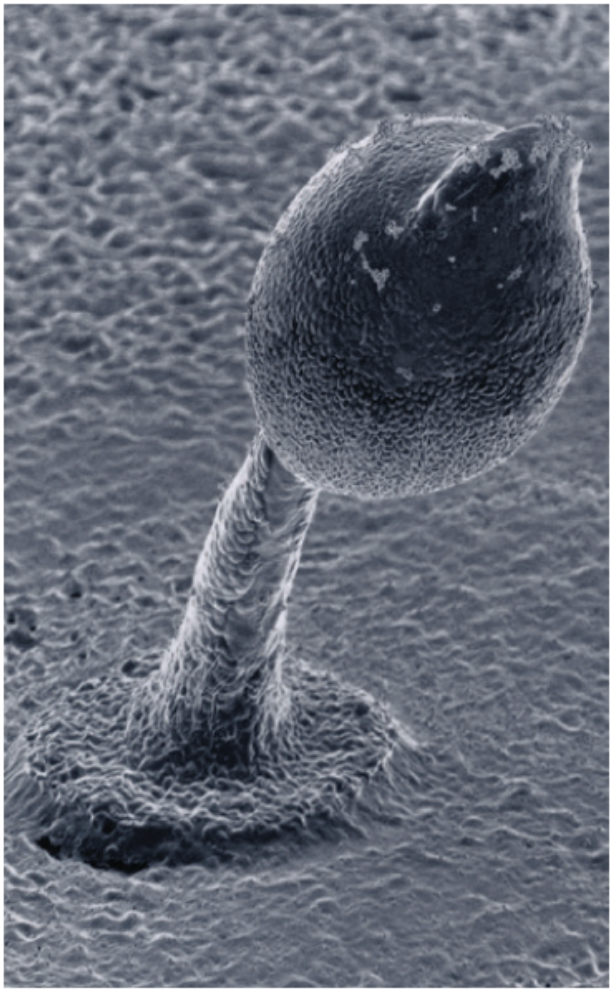
41. CONNECT Ribosomal RNAs are the most abundant RNAs in the cell. In experiments looking at levels of gene expression in different cell types, rRNAs are generally removed before carrying out RNA sequencing. Why might this be a useful step in these experiments? Why are researchers often less interested in rRNA than in other types of RNA?

42. CONNECT What might be some advantages of using CRISPR-Cas (see Chapter 19) for carrying out mutagenesis screens?

Section 20.3

43. CONNECT Horizontal gene transfer is common in bacteria and has played a role in bacterial genome evolution. Explain how horizontal gene transfer may occur through transformation and transduction. For information about transformation and transduction, see Chapter 9.

44. DATA ANALYSIS *Dictyostelium discoideum* is a soil-dwelling social amoeba: much of the time, the organism consists of single, solitary cells, but during times of starvation, amoebae come together to form aggregates that have many characteristics of multicellular organisms. Biologists have long debated whether *D. discoideum* is a unicellular or a multicellular organism. In 2005, its genome was completely sequenced. The accompanying table lists some genomic characteristics of *D. discoideum* and other eukaryotes (L. Eichinger et al. 2005. *Nature* 435:43–57).



David Scharf/Science Source.

Dictyostelium discoideum.

a. On the basis of the organisms other than *D. discoideum* listed in the table, what are some differences in genome characteristics between unicellular and multicellular organisms?

b. On the basis of these data, do you think the genome of *D. discoideum* is more like those of unicellular eukaryotes, or more like those of multicellular eukaryotes? Explain your answer.

Table for Problem 44: Genomic characteristics of *D. discoideum* and other eukaryotes

Feature	*D. discoideum*	*P. falciparum*	*S. cerevisiae*	*A. thaliana*	*D. melanogaster*	*C. elegans*	*H. sapiens*

Organism	Amoeba	Protozoan	Yeast	Plant	Fruit fly	Nematode	Human
Cellularity	?	Uni	Uni	Multi	Multi	Multi	Multi
Genome size (millions of base pairs)	34	23	13	125	180	103	2851
Number of genes	12,500	5268	5538	25,498	13,676	19,893	22,287
Average gene length (bp)	1756	2534	1428	2036	1997	2991	27,000
Genes with introns (%)	69	54	5	79	38	5	85
Mean number of introns	1.9	2.6	1.0	5.4	4.0	5.0	8.1
Mean intron size (bp)	146	179	nd*	170	nd*	270	3365
Mean G + C (exons)	27%	24%	28%	28%	55%	42%	45%

*nd = Not determined.

45. *How do the following genomic features of prokaryotic organisms compare with those of eukaryotic organisms? How do they compare among eukaryotes?
    a. Genome size
    b. Number of genes
    c. Gene density (bp/gene)
    d. Number of exons

46. DATA ANALYSIS A group of scientists sequenced the genomes of 12 species of *Drosophila* (*Drosophila* 12 Genomes Consortium. 2007. *Nature* 450:203–218). Data on genome size and number of protein-encoding genes from this study are given

in the accompanying table (see top right of this page). Plot the number of protein-encoding genes as a function of genome size for the 12 species of *Drosophila*. Is there a relation between genome size and number of genes in fruit flies? How does this compare with the relation between genome size and number of genes across all eukaryotes?

Table for Problem 46: Characteristics of 12 *Drosophila* species genomes

Species	Genome Size (Millions of Base Pairs)	Number of Protein-Encoding Genes
D. melanogaster	200	13,733
D. simulans	162	15,983
D. sechellia	171	16,884
D. yakuba	190	16,423
D. erecta	135	15,324
D. ananassae	217	15,276
D. pseudoobscura	193	16,363
D. persimilis	193	17,325
D. willistoni	222	15,816
D. virilis	364	14,680
D. mojavensis	130	14,849

D. grimshawi	231	15,270

47. CONNECT The remains of transposable elements (see Chapter 18) make up a substantial proportion of some eukaryotic genomes. For example, 45% of the DNA in the human genome is derived from transposable elements. Explain why transposable element sequences are so numerous in eukaryotic genomes.

Section 20.4

48. A scientist determines the complete genomes and proteomes of a liver cell and a muscle cell from the same person. Would you expect bigger differences in the genomes or in the proteomes of these two cell types? Explain your answer.

49. CONNECT Most organisms produce many more types of proteins than there are genes in their genome. Explain how there can be more proteins than genes, when all proteins must be encoded by DNA sequences.

CHALLENGE QUESTIONS

Section 20.1

50. DATA ANALYSIS The genome of *D. melanogaster*, a fruit fly, was sequenced in 2000. However, this "completed" sequence did not include most heterochromatin regions. The heterochromatin was not sequenced until 2007 (R. A. Hoskins et al. 2007. *Science* 316:1625–1628). Most completed genome sequences do not include heterochromatin. Why is heterochromatin usually not sequenced in genome-sequencing projects? (Hint: See Chapter 11 for a more detailed discussion of heterochromatin.)

51. In metagenomic studies, a comparison of ribosomal RNA sequences is often used to determine the number of different species present. What are some characteristics of ribosomal sequences that make them useful for determining what species are present?

52. *Some synthetic biologists have proposed creating an entirely new, free-living organism with a minimal genome, the smallest set of genes that allows for replication of the organism in a particular environment. This genome could be used to design and create, from scratch, novel organisms that might perform specific tasks, such as the breakdown of toxic materials in the environment.
    a. How might the minimal genome required for life be determined?
    b. What, if any, social and ethical concerns might be associated with the construction of an entirely new organism with a minimal genome?

💬 ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 20.1

1. Increasingly, whole-genome sequencing of individuals is being done to help identify and treat medical conditions. Genome sequencing invariably identifies a number of variations, some common and some rare, that might be clinically relevant. For example, suppose a person had their genome sequenced to help determine their risk for cardiovascular disease and, just by chance, the sequence revealed that they carry one or more variants that predispose them to cancer or Alzheimer disease. Does the sequencing laboratory or physician have an obligation to report this finding, which was not the purpose of the sequencing and which the patient did not request? What about reporting variants for which no or limited information can be provided about their clinical significance? Does the answer to this question differ for sequencing done on children?

2. If “the” human genome sequence does not really exist (see What Exactly Is the Human Genome? in this chapter), can you think of better ways in which we might represent the human genome? Propose some possibilities.

3. As pointed out in the text, you and a complete stranger are 99.9% identical in DNA sequence. But you also differ at more than 3 million base pairs. Is this a large or a small difference? What are some of the consequences of these similarities and differences?

4. What are some possible research questions and practical applications that could be addressed by creating organisms with artificial chromosomes and synthetic genomes? What might be some potential safety, environmental, social, and ethical concerns about creating organisms with synthetic genomes?

Section 20.3

5. Researchers systematically replaced 414 essential genes in yeast with similar genes from humans. Almost half of these transplants (47%) were successful: cells with the humanized gene were able to function and grow. What does this observation tell us about differences between yeast and humans? How might this information be used?

6. The Japanese canopy plant (*Paris japonica*) has one of the largest of all eukaryotic genomes, with approximately 150 billion base pairs, about 50 times the size of the human genome. In contrast, the bladderwort *Utricularia gibba* has one of the smallest plant genomes, with only 82 million base pairs. What predictions can you make about the genomes of these two species?

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Use a concept map to outline some of the reasons for conducting the Earth BioGenome Project and some of the challenges it faces.

Section 20.1

2. Create a concept map that summarizes the different aspects of structural genomes. Include the following terms in your concept map: structural genomics, genetic maps, recombination rates, physical maps, restriction mapping, map-based sequencing, whole-genome shotgun sequencing, single-nucleotide polymorphisms, haplotypes, genome-wide association studies, metagenomics.

Section 20.2

3. Draw a concept map that includes the following terms and concepts for functional genomics: functional genomics, transcriptome, proteome, homologous genes, orthologs, paralogs, microarrays, RNA sequencing, scRNA-seq, mutagenesis screen.

Section 20.3

4. Create a concept map that compares and contrasts the characteristics of prokaryotic and eukaryotic genomes, such as genome size, number of genes, relationship between gene size and gene number, organization, and other features.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 21

Epigenetics

Keystone-France/Getty Images.

The Dutch Hunger Winter, a severe famine in the Netherlands at the end of World War II, had long-term effects on people who were conceived and developed prenatally during the famine. These effects are thought to be due to epigenetic alterations. Shown here is a young girl eating after the famine ended.

Epigenetics and the Dutch Hunger Winter

In 1944, near the close of World War II, Allied armies marched north into the Netherlands to liberate the country from German occupation. The Allied advance, however, came to a halt at the Waal and Rhine rivers. To help the Allied cause, the Dutch people had carried out a railway strike; in retaliation, the German army blocked all food supplies to cities in the north of the country, and the Dutch people starved. From October through April, no food reached the area, and food supplies quickly ran out. People subsisted on bread and potatoes. In November 1944, food rations provided fewer than 1000 calories per person per day; by April 1945, many people were getting fewer than 500 calories per day. Thousands died. The famine ended in May 1945, when Allied armies liberated the entire country.

Known as the Dutch Hunger Winter, this terrible episode of intense starvation provides a natural experiment for examining the consequences of famine for humans. Researchers have been particularly interested in the long-term effects of starvation on people who were conceived and who developed prenatally during the famine. Numerous studies have examined these individuals, who are now adults. What the researchers found was remarkable: the caloric restriction that these people experienced while in the womb had life-long consequences. For example, men who were conceived and went through early development during the famine

were twice as likely to be obese by age 18. Both men and women who underwent early development during the famine were more likely to be obese by age 50 and more likely to die from stroke and cardiovascular disease. They also experienced more cognitive decline in later life.

Scientists believe that these long-term effects are due to epigenetic alterations: modifications of chromatin and DNA that alter gene expression but do not change the base sequence of the DNA. One common epigenetic change is DNA methylation, the addition of methyl groups to cytosine bases in the DNA. Geneticists examined DNA from individuals who were conceived during the Dutch Hunger Winter and compared it with DNA from their same-sex siblings conceived at other times. They examined 15 loci that are involved in growth and metabolic disease. Of these loci, 6 showed significant differences in methylation patterns. At some loci, methylation was higher among the famine-exposed individuals, and at other loci, methylation was lower.

These results show that an early life event—caloric restriction during prenatal development—can bring about alterations in chromatin structure that have long-term consequences. Studies on rodents show that the phenotypic effects of nutrition can carry over for several generations. As this chapter explains, epigenetic alterations can be passed from cell to cell and sometimes from generation to generation. Epigenetic inheritance was not envisioned by Mendel nor, until recently, by most modern geneticists, but

epigenetic processes appear to play an important role in the inheritance of many phenotypes. Today, the study of epigenetics is the focus of intensive research.

A question that this study raises is why limited calories during prenatal development would *increase* adult obesity and risk of dying from cardiovascular disease. One might expect just the opposite, that nutritional stress during fetal development would decrease adult body weight. Evolutionary biologists have proposed an explanation for this relation, which has also been observed in other studies. Termed the *thrifty phenotype hypothesis*, it proposes that when environmental conditions are poor during fetal development, they are usually likely to persist and to be poor in adult life as well. The fetus responds by developing a metabolically thrifty phenotype—eating as much as possible when food is available, minimizing energy expenditure, and lowering metabolic rate—because the prenatal environment predicts that there will be little food available in the future. This strategy was probably advantageous in the distant past, before agriculture, but it often backfires in modern society. Eating all you can, minimizing energy expenditure, and hoarding calories when food is plentiful often leads to obesity, heart disease, and diabetes.

THINK-PAIR-SHARE

- Among people who were prenatally exposed to famine during the Dutch Hunger Winter, obesity and increased risk of cardiovascular disease are seen only in those who

were in early gestational stages during the famine; those individuals who were in the last trimester of prenatal development do not show these effects. Propose an explanation for why those in early stages of development were affected but not those in later stages.
- What are some practical implications of the finding that caloric restriction during fetal development has long-term negative effects on adult body weight, cardiovascular disease, and later-in-life cognitive decline?

This chapter is about epigenetics, which is the explanation for the effect of famine on the future health of the people who were conceived during the Dutch Hunger Winter. We begin by discussing the origin of the term *epigenetics* and what the term encompasses today. We then review the types of changes in chromatin that can occur and the major processes that alter chromatin structure. We also take a look at how changes in chromatin structure might be passed on to future cells and future generations. We then look at a number of epigenetic effects, including effects on cell differentiation, X inactivation, genomic imprinting, effects on monozygotic twins, paramutation, behavioral effects, the effects of chemicals, and metabolic effects. We end the chapter by discussing efforts to map the genome-wide pattern of epigenetic marks—the epigenome.

21.1 What Is Epigenetics?

The term *epigenetics* is based on the Greek root *epi*, meaning "over" or "above"; the term *epigenetics* has come to represent the inheritance of variation above and beyond differences in DNA sequence. *Epigenetics* usually refers to the phenotypes and processes that are transmitted to other cells and sometimes to future generations but that are not the result of differences in the DNA base sequence (**Figure 21.1**). Many epigenetic effects are caused by changes in gene expression that result from alterations in chromatin structure or other aspects of DNA structure, such as DNA methylation. One definition of an epigenetic trait is a stably inherited phenotype resulting from changes in chromatin without alterations in the DNA sequence. Some have broadened the definition of epigenetics to refer to any alteration of chromatin or DNA structure that affects gene expression. Here, we will use *epigenetics* to refer to changes in gene expression or phenotype that are potentially heritable but that do not alter the underlying DNA base sequence.

Courtesy of Enrico Coen.

21.1 Differences in flower shape in toad flax (*Linaria vulgaris*) are due to epigenetic variation. The wild-type flower (left) and peloric mutant (right) are identical in their DNA sequence but differ in the methylation of their DNA.

Description
The petals of the first flower forms a funnel shaped structure. The second flower has disk-shaped structure on top and a star-like structure at bottom.

Many epigenetic changes are stable, persisting across cell divisions or even generations. However, epigenetic alterations can also be influenced by environmental factors. For example, environmental stress has been shown to alter methylation of the rat *Bdnf* gene, which encodes a growth factor that plays an important role in brain development. DNA methylation has been tied to the expression of genes and the phenotypes they produce. As we will see, altered DNA methylation is capable of being replicated across cell division, resulting in progeny with the new phenotype; however, there is no corresponding difference in the DNA base sequence of individuals that “inherit” the new phenotype. The fact that epigenetic traits may

be induced by environmental effects and transmitted to future generations has been interpreted by some to mean that, through epigenetics, genes have memory—that environmental factors acting on individuals can have effects that are transmitted to future generations, as was seen with the effect of diet on obesity and cardiovascular disease in the introduction to this chapter. Epigenetics has been called "inheritance, but not as we know it."

Epigenetics is providing an explanation for how changes outside the DNA sequence can influence the phenotype and how those changes can be heritable. It is also making important contributions to the study of behavior, environmental science, cancer, neurobiology, and pharmacology. › **TRY PROBLEM 2**

THINK-PAIR-SHARE Question 1

CONCEPTS

Epigenetic effects are phenotypes that are passed to other cells and sometimes to future generations but that are not the result of differences in the DNA base sequence. The study of epigenetics is making important contributions to many areas of biology.

21.2 Several Molecular Processes Lead to Epigenetic Changes

Epigenetics alters the expression of genes; these alterations are stable enough to be transmitted through mitosis (and sometimes meiosis) but can also be changed. Most evidence suggests that epigenetic effects are brought about by physical changes in chromatin structure. In Chapter 11, we considered a number of chemical changes in DNA and histone proteins that affect chromatin structure, including DNA methylation, modification of histone proteins, and repositioning of nucleosomes. In Chapter 17, we discussed the role that these alterations have on the expression of genes. These chromatin changes are thought to play a role in epigenetic traits. Chapter 14 discussed small RNA molecules, some of which play an important role in bringing about epigenetic changes.

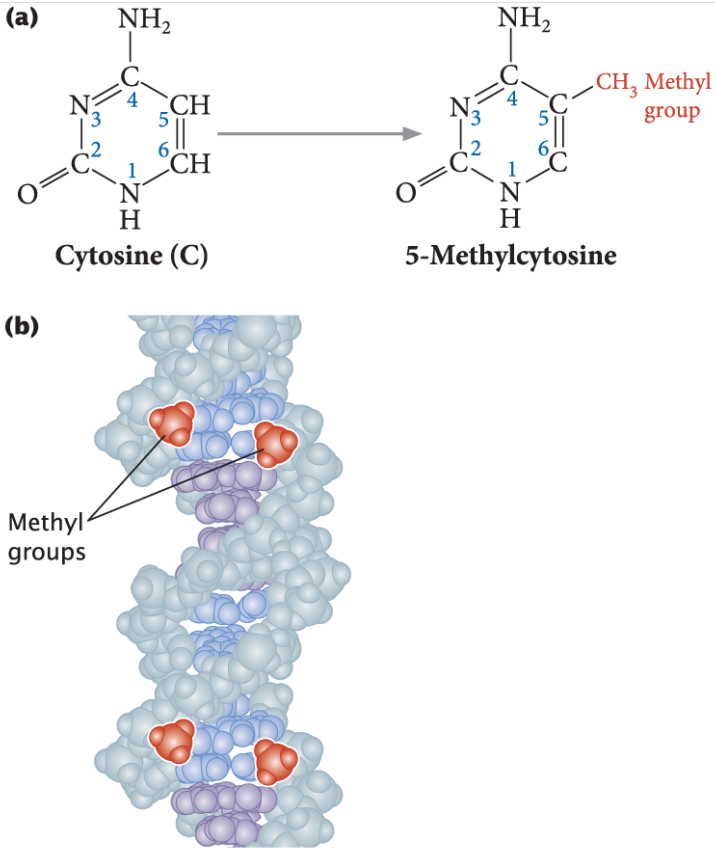
In this section, we consider three types of molecular mechanisms that alter chromatin structure and underlie many epigenetic phenotypes: (1) changes in patterns of DNA methylation; (2) chemical modifications of histone proteins; and (3) RNA molecules that affect chromatin structure and gene expression.

CONCEPTS

Many epigenetic phenotypes are the result of alterations in chromatin structure, mediated through three major processes: DNA methylation, histone modifications, and RNA molecules.

DNA Methylation

The best-understood mechanism of epigenetic change is methylation of DNA. DNA methylation refers to the addition of methyl groups to the nucleotide bases. In eukaryotes, the predominant type of DNA methylation is the methylation of cytosine to produce 5-methylcytosine (**Figure 21.2a**). As discussed in Section 17.2, DNA methylation is often associated with the repression of transcription.



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21.2 DNA methylation is a common epigenetic modification of chromatin. (a) Cytosine bases are often modified to form 5-methylcytosine. (b) Three-dimensional structure of DNA showing methylation of CpG dinucleotides.

Description

Part “a” shows the conversion of cytosine into 5-Methylcytosine. The structure of cytosine has a pyrimidine ring, with C 2 double bonded to an oxygen atom. C 4 is bonded to an amino group (N H 2). The structure of pyrimidine ring is as follows: N H bond C bond N double bond C bond C H double bond C H. A forward arrow leads to 5-Methylcytosine. It shows the structure of cytosine, in which the hydrogen bonded to C 5 is replaced by a methyl group (C H 3). Part “b” shows a 3-D structure of D N A with methyl groups in between.

DNA methylation often occurs on cytosine nucleotides that are immediately adjacent to guanine nucleotides, together referred to as CpG dinucleotides (where p represents the phosphate group that connects the C and G nucleotides). In CpG dinucleotides, cytosine nucleotides on the two DNA strands are diagonally across from each other. Usually both cytosine bases are methylated, so that methyl groups occur on both DNA strands, as shown below and in **Figure 21.2b**.

$$5'-\overset{\text{m}}{\text{C}}\,\text{G}-3'$$
$$3'-\text{G}\,\underset{\text{m}}{\text{C}}-5'$$

In plants, DNA methylation also occurs at CpNpG and CpNpN trinucleotides, where N represents a nucleotide with any base except G.

Some DNA regions have many CpG dinucleotides and are referred to as CpG islands. In mammalian cells, CpG islands are often located in or near the promoters of genes. These CpG islands are usually not methylated when genes are being actively transcribed. However, methylation of CpG islands near a gene leads to the repression of transcription.

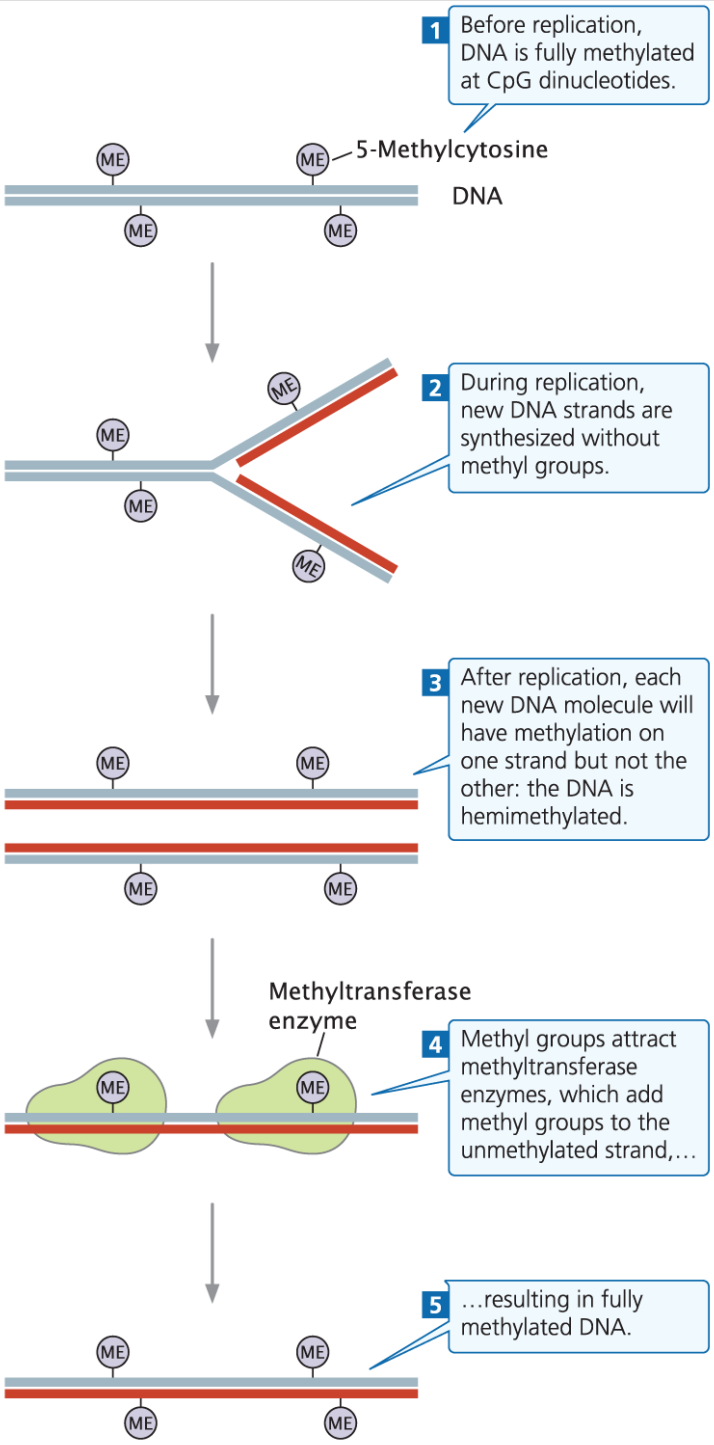
Cells repress and activate genes by methylating and demethylating cytosine bases. Enzymes called DNA methyltransferases methylate DNA by adding methyl groups to cytosine bases to create 5-

methylcytosine. Other enzymes, called demethylases, convert 5-methylcytosine back to cytosine (see Section 17.2).

MAINTENANCE OF METHYLATION

The fact that epigenetic changes are passed to other cells and sometimes to future generations means that the changes in chromatin structure associated with epigenetic phenotypes must be faithfully maintained when chromosomes replicate. How are epigenetic changes retained and replicated through the process of cell division?

As shown above, when CpG dinucleotides are methylated, the two methylated cytosine bases sit diagonally across from each other on opposite strands. Before replication, cytosine bases on both strands are methylated (**Figure 21.3**). Immediately after semiconservative replication, the cytosine base on the template strand is methylated, but the cytosine base on the newly replicated strand is unmethylated. Methyltransferase enzymes recognize the hemimethylated state of CpG dinucleotides and add methyl groups to the unmethylated cytosine bases, resulting in two new DNA molecules that are fully methylated. In this way, the methylation pattern of DNA is maintained across cell division. **› TRY PROBLEM 24**



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21.3 DNA methylation is stably maintained through DNA replication.

Description

The illustration starts with a D N A with four 5-Methylcytosine, two on the upper strand and two on the bottom. A caption reads, “Before replication, D N A is fully methylated at C p G dinucleotides.” This D N A unwinds at the right end and newly synthesized strands are shown. A caption reads, “During replication, new D N A strands are synthesized without methyl groups.” Next, it leads to two template strands with methyl groups with their two newly synthesized strands. A caption reads, “After replication, each new D N A molecule will have methylation on one strand but not the other: the D N A is hemimethylated.” One D N A is shown then with a template strand with two methyl groups and a newly synthesized strand with no methyl group. Methyltransferase enzyme attaches to both the methyl groups. A caption reads, “Methyl groups attract methyltransferase enzymes, which add methyl groups to the unmethylated strand, resulting in fully methylated D N A.” Then, the other strand also shows two methyl groups.

DNA METHYLATION IN HONEYBEES

A remarkable example of epigenetics is seen in honeybees. Queen bees and worker bees are both female, but the resemblance ends there. The queen is large and develops functional ovaries, whereas workers are small and sterile (Figure 21.4). The queen goes on a mating flight and then spends the rest of her life reproducing, whereas workers spend all their time collecting nectar and pollen, tending the queen, and raising her offspring. In spite of these differences in their anatomy, physiology, and behavior, queens and workers are genetically the same; both develop from ordinary eggs. How they differ is in diet: worker bees produce and feed a few female larvae a special substance called royal jelly, which causes these larvae to develop as queens. Other larvae are fed ordinary bee food, and they develop as workers. This simple difference in diet greatly affects gene expression, causing different genes to be

activated in queens and in workers, and resulting in very different sets of phenotypic traits.

WILDLIFE GmbH/Alamy.

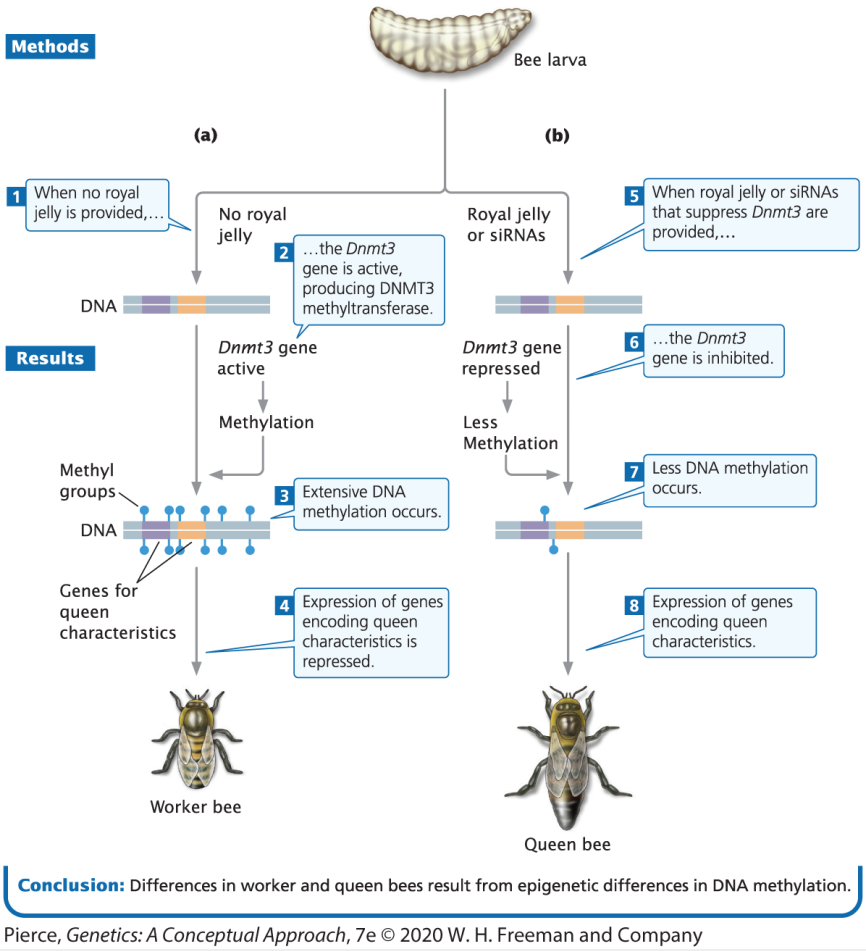
21.4 Epigenetic changes are responsible for differences in the phenotypes of honeybee (*Apis mellifera*) queens (left) and workers (right).

How royal jelly affects gene expression has long been a mystery, but research now suggests that it changes an epigenetic mark. In 2008, Ryszard Kucharski and his colleagues demonstrated that royal jelly silences the expression of a key gene called *Dnmt3*, whose product normally adds methyl groups to DNA (**Figure 21.5**). With *Dnmt3* shut down, bee DNA is less methylated, and many genes that are normally silenced in workers are expressed, leading to the development of queen characteristics. Kucharski and his colleagues demonstrated the importance of DNA methylation in queen development by injecting bee larvae with small interfering RNAs (siRNAs; see Section 14.5) that specifically inhibited the expression of *Dnmt3*. These larvae had lower levels of DNA methylation, and many developed as queens with fully functional ovaries. This experiment demonstrated

that royal jelly brings about epigenetic changes (less DNA methylation), which are transmitted through cell division and modify developmental pathways, eventually leading to a queen bee.

› **TRY** [PROBLEM 23]

THINK-PAIR-SHARE [Question 2]



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21.5 Phenotypic differences in queen and worker honeybees result from epigenetic differences in DNA methylation. Royal jelly suppresses *Dnmt3*, which normally methylates DNA, leading to the expression of genes that encode characteristics of the queen.

Description

The question in this experiment is: How does royal jelly produce phenotypic differences in worker and queen bees? The methods section shows a bee larva. It leads to two branches labeled “a” and “b”. Part “a” shows no royal jelly. It shows a D N A with two genes represented by blue and yellow. A caption reads, “When no royal jelly is provided, the D n m t 3 gene is active, producing D N M T 3 methyltransferase.” The results section shows methylation in active D n m t 3 gene, which adds to the D N A. It shows twelve methyl groups on the D N A. The two colored regions on the D N A are labeled genes for queen characteristics. A caption reads, “Extensive D N A methylation occurs.” Another caption reads, “Expression of genes encoding queen characteristics is repressed.” The illustration then leads to a worker bee. Part “b” shows royal jelly or s i R N A’s. It also shows a D N A with two genes represented by blue and yellow bands. A caption reads, “When royal jelly or s i R N A’s that suppress D n m t 3 are provided, the D n m t 3 gene is inhibited.” The results section shows less methylation in repressed D n m t 3 gene, which adds to the D N A. It shows only two methyl groups on the D N A. A caption reads, “Less D N A methylation occurs.” Another caption reads, “Expression of genes encoding queen characteristics.” The illustration then leads to a queen bee. Text at the bottom reads, “Conclusion: Differences in worker and queen bees result from epigenetic differences in D N A methylation.”

REPRESSION OF TRANSCRIPTION BY DNA METHYLATION

How does DNA methylation suppress gene expression? The methyl group of 5-methylcytosine sits within the major groove of the DNA molecule, which is recognized by many DNA-binding proteins. The presence of the methyl group in the major groove inhibits the binding of transcription factors and other proteins required for transcription to occur. The 5-methylcytosine also attracts certain

proteins that directly repress transcription. In addition, DNA methylation attracts histone deacetylase enzymes, which remove acetyl groups from the tails of histone proteins, altering chromatin structure in a way that represses transcription (see Section 17.2).

CONCEPTS

Cytosine bases are often methylated to form 5-methylcytosine, which is associated with the repression of transcription. DNA methylation is stably maintained through replication by methyltransferase enzymes that recognize the hemimethylated state of CpG dinucleotides and add methyl groups to the unmethylated cytosine bases.

✔ CONCEPT CHECK 1

Which of the following is true of CpG islands?

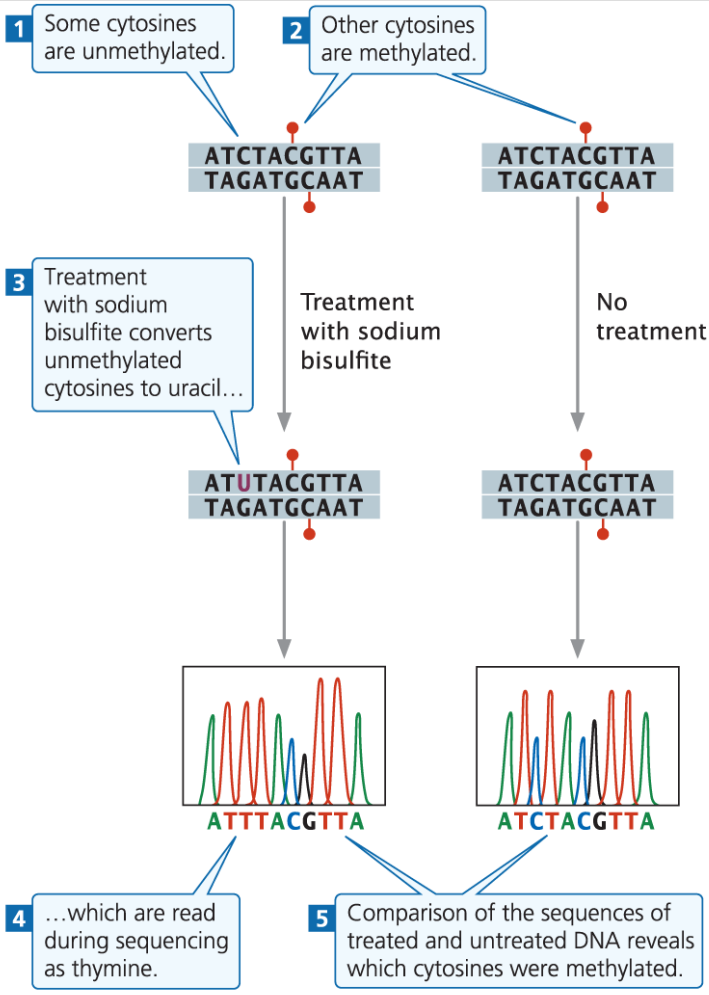
a. They are methylated near promoters of actively transcribed genes.
b. They are unmethylated near promoters of actively transcribed genes.
c. Acetylation of CpG islands leads to the repression of transcription.
d. CpG islands code for RNA molecules that activate transcription.

DETECTING DNA METHYLATION

A number of techniques have been developed for examining levels of DNA methylation. One set of techniques relies on restriction endonucleases, enzymes that make double-stranded cuts in the DNA at specific base sequences (see Section 19.2). Some restriction enzymes are sensitive to methylation and will not cut a sequence that contains 5-methylcytosine, whereas other restriction enzymes

are insensitive to methylation. By cutting DNA with enzymes that are sensitive to methylation and with enzymes that are not, and then analyzing the resulting fragments, overall patterns of methylation can be determined.

A more precise and widely used technique for analyzing DNA methylation is bisulfite sequencing (**Figure 21.6**). In this technique, genomic DNA is first treated with sodium bisulfite, which chemically converts unmethylated cytosine to uracil. Uracil is then detected as thymine during sequencing. However, 5-methylcytosine is not chemically altered by treatment with bisulfite, and it is detected as cytosine during sequencing (see Section 19.5 for a discussion of DNA sequencing). By sequencing genomic DNA with and without bisulfite treatment, researchers are able to determine the locations of all copies of 5-methylcytosine in the DNA.



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21.6 Bisulfite sequencing can be used to determine the locations of 5-methylcytosines.

Description

The illustration shows two similar D N A sequences as follows: First strand, A T C T A methylated C G T T A; second strand, T A G A T G methylated C A A T. Two captions read as follows: Some cytosines are unmethylated. Other cytosines are methylated. The first D N A sequence is treated with sodium bilsulfite and the second undergoes no treatment. The first D N A sequence is changed and now shows the following sequence: First strand, A T U T A methylated C G T T A; second strand, T A G A T G methylated C A A T. A caption reads, “Treatment with sodium bisul¬fite converts unmethylated cytosines to uracil, which are read during sequencing as thymine.” The second D N A sequence

remains unchanged. Each D N A sequence shows a graph with several curves of unequal height and sequence is written beneath it. The first graph has the following sequence: A T T T A C G T T A. The second graph has the following sequence: A T C T A C G T T A. A caption reads, “Comparison of the sequences of treated and untreated D N A reveals which cytosines were methylated.”

CONCEPTS

DNA methylation can be detected by cutting the DNA with restriction endonucleases that are sensitive to DNA methylation, as well as by bisulfite sequencing.

Histone Modifications

Epigenetic changes can also occur through modification of histone proteins. In eukaryotic cells, DNA is complexed to histone proteins in the form of nucleosomes, which are the basic repeating units of chromatin structure (see Chapter 11). Modifications of histones include the addition of phosphates, methyl groups, acetyl groups, and ubiquitin. Many of these modifications take place in the positively charged tails of the histone proteins, which interact with the DNA and affect chromatin structure. These modifications can occur at different amino acids on different histones and can create more than 100 unique potential changes in the histones. Many of these modifications alter chromatin structure and affect the transcription of genes (see Chapter 17). They may also serve as

binding sites for proteins, such as transcription factors that are required for transcription.

The addition of acetyl groups to amino acids in the histone tails (histone acetylation) generally destabilizes chromatin structure, causing it to assume a more open configuration; this addition is associated with increased transcription (see Figure 17.2). The addition of methyl groups to histones (histone methylation) also alters chromatin structure, but the effect varies depending on the specific amino acid that is methylated; some types of histone methylation are associated with increased transcription, and other types are associated with decreased transcription. For example, the addition of three methyl groups to lysine 4 in the H3 histone (H3K4me3; K stands for lysine) is often found near transcriptionally active genes. Methylation of lysine 36 in the H3 histone (H3K36me3) is also associated with increased transcription. On the other hand, the addition of three methyl groups to lysine 9 in H3 (H3K9me3) or to lysine 20 in histone 4 (H4K20me3) is associated with the repression of transcription. Many additional histone modifications, as well as changes to DNA that do not involve the base sequence, have been shown to be associated with the level of transcription. These types of modifications have been called **epigenetic marks**.

Histone modifications are added and removed by special proteins. The Polycomb group (PcG) is a large group of proteins that repress transcription by modifying histones. These modifications alter chromatin structure so that the DNA is not accessible to

transcription factors, RNA polymerase, and other proteins required for transcription. For example, Polycomb repressive complex 2 (PRC2) adds two or three methyl groups to lysine 27 of histone H3, creating the H3K27me3 epigenetic mark, which represses transcription.

Many of the enzymes and proteins that produce epigenetic marks cannot bind to specific DNA sequences by themselves. Thus, they must be recruited to specific targets on the chromosome. Sequence-specific transcription factors, preexisting chromatin marks, and noncoding RNA molecules serve to recruit histone-modifying enzymes to specific sites.

Research indicates that single histone modifications, such as those mentioned here, do not individually determine the transcriptional activity of a gene. Rather, it is the combined presence of multiple epigenetic marks that determines the activity level. There is also considerable "crosstalk" between epigenetic marks: one histone mark may affect whether additional marks occur nearby and how they function. Crosstalk occurs because histone modifications attract enzymes and proteins that modify other histones.

Histone modifications not only affect transcription but can also influence other molecular processes, such as DNA repair and cell cycle checkpoint signaling (see pp. 713–715 in Chapter 23). For example, ubiquitination of histone H2B is required for the repair of double-strand breaks in DNA. This modification leads to other

histone modifications, such as methylation of lysine 79 of histone H3 (H3K79me); these modifications alter chromatin structure and allow proteins that repair double-strand breaks to access the DNA.

MAINTENANCE OF HISTONE MODIFICATIONS

The process by which histone modifications are maintained across cell division is not as well understood as that of DNA methylation. There is no universal mechanism for maintaining histone modifications; different types of modifications are undoubtedly maintained by different mechanisms.

Several models have been proposed to explain how histone modifications are faithfully transmitted to daughter cells. During the process of DNA replication, nucleosomes are disrupted, and the original histone proteins are distributed randomly between the two new DNA molecules. Newly synthesized histones are then added to complete the formation of new nucleosomes (see Section 12.4). Some models assume that after replication, the epigenetic marks remain on the original histones, and that these marks recruit enzymes that make similar changes in the new histones. These enzymes are said to “read and write”: they recognize (read) the epigenetic modifications, bind to them, and then modify (write) nearby histones that are lacking the modification. For example, PRC2, which adds the H3K27me3 epigenetic mark to histones, preferentially targets histones in chromatin that already contains an H3K27me3 mark, ensuring that any new nucleosomes that are added

after replication also become methylated. In this way, the histone modifications can be maintained across cell division. An alternative model, supported by experimental evidence in *Drosophila* embryos, proposes that the epigenetic marks are lost during replication, but that the enzymes that bring about histone modifications remain attached to the original histones during replication and reestablish the marks on the original and new histones after replication is completed.

DETECTING HISTONE MODIFICATIONS

Histone modifications can be detected by breaking the chromatin into fragments and applying an antibody specific to a particular histone modification in a process called chromatin immunoprecipitation (abbreviated ChIP; see Section 17.2). The antibody mediates the precipitation of chromatin fragments with the histone modification, causing them to separate from chromatin fragments without the modification. The histones are then removed by digestion with an enzyme that degrades protein but not DNA. The genomic location of the precipitated DNA fragment with which the histone was associated is then determined. In a technique called ChIP-Seq, the fragments are sequenced and their genomic locations are determined by comparing the sequence with a reference genome (see Figure 17.4). This technique provides information about where in the genome histone modifications occur.

CONCEPTS

Modifications of histone proteins, including the addition of methyl groups, acetyl groups, phosphates, and ubiquitin, alter chromatin structure. Some of these modifications are passed to daughter cells during cell division and to future generations. Histone modifications can be detected with chromatin immunoprecipitation.

Epigenetic Effects of RNA Molecules

Evidence increasingly demonstrates that RNA molecules play an important role in bringing about epigenetic effects. The first discovered and still best-understood example of RNA mediation of epigenetic change is X inactivation (mentioned in Section 4.3), in which a long noncoding RNA called *Xist* suppresses transcription on one of the X chromosomes in female mammals. Another example involves paramutation in corn, in which an epigenetically altered allele, by means of siRNAs, induces a change in another allele that then gets transmitted to future generations. Both of these examples will be discussed in Section 21.3.

Various mechanisms are involved in epigenetic changes produced by RNA molecules. In the case of X inactivation, the *Xist* RNA coats one X chromosome and then attracts PRC2, which deposits methyl groups on lysine 27 of histone H3, creating the H3K27me3 epigenetic mark, which alters chromatin structure and represses transcription.

Other examples of RNA-associated epigenetic phenotypes are produced by siRNA molecules that silence genes and transposable elements (see Section 18.4) by directing DNA methylation or histone modifications to specific DNA sequences. In addition, research has demonstrated that epigenetic processes such as methylation and histone modification influence the expression of microRNAs (see Section 14.5), which in turn play an important role in regulating other genes. MicroRNAs also control the expression of genes that produce epigenetic effects, such as those encoding enzymes that methylate DNA and modify histone proteins. How RNA-based epigenetic changes are maintained across cell divisions is less clear, although some apparently involve small RNAs that are transmitted through the cytoplasm. **Table 21.1** lists some of the molecular processes that lead to epigenetic changes and their effect on gene expression.

TABLE 21.1 Molecular processes that produce epigenetic changes

Process	Effect on Gene Expression
DNA methylation	Represses transcription
Acetylation of histone proteins	Stimulates transcription
Methylation	Represses or stimulates transcription

of histone proteins	depending on which amino acids are methylated
RNA molecules	May repress or stimulate transcription

CONCEPTS

RNA molecules bring about modifications of chromatin by a variety of processes.

✔ CONCEPT CHECK 2

Which of the following is not a major mechanism of epigenetic change?

a. DNA methylation
b. Alteration of a DNA base sequence in a promoter
c. Histone acetylation
d. Action of RNA molecules

21.3 Epigenetic Processes Affect Development

Initially, epigenetic mechanisms were thought to play a role in a small number of unusual phenotypes. However, research has now revealed that epigenetics underlies an impressive and ever-increasing array of biological phenomena. In this section, we look at epigenetic modifications that influence developmental processes.

Epigenetic Changes Associated with Cell Differentiation

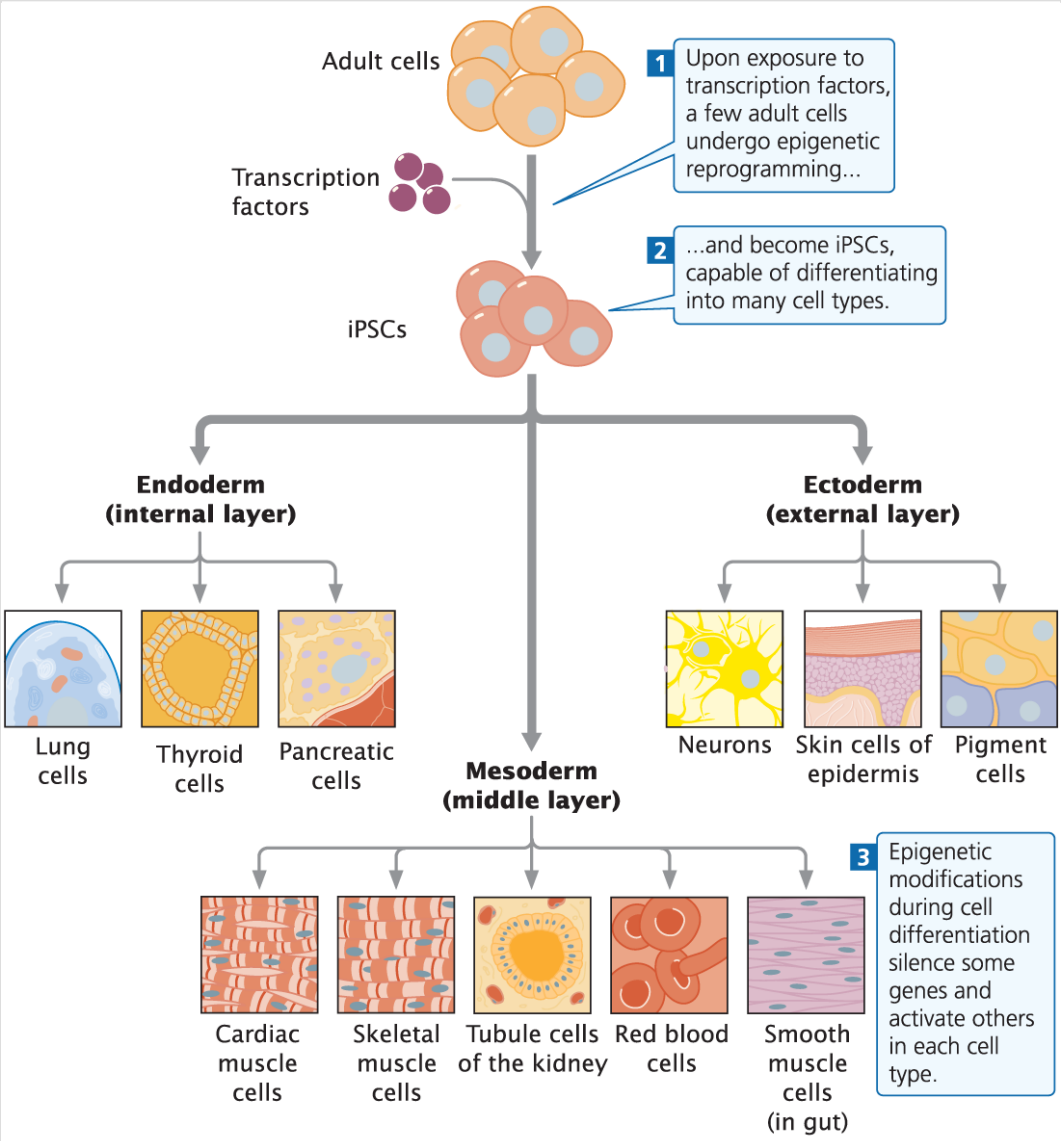
All cells in the human body are genetically identical, and yet different cell types exhibit remarkably different phenotypes—a nerve cell is quite distinct in its shape, size, and function from an intestinal cell. These differences in phenotypes are stable and are passed from one cell to another, despite the fact that the DNA sequences of all the cells are the same.

Stem cells are undifferentiated cells that are capable of forming every type of cell in an organism, a property referred to as **pluripotency**. As a stem cell divides and gives rise to a more specialized type of cell, the gene-expression program of the cell becomes progressively fixed, so that each particular cell type

expresses only those genes necessary to carry out the functions of that cell type. Though the control of these cell-specific expression programs is not well understood, changes in DNA methylation and chromatin structure clearly play important roles in silencing some genes and activating others.

Stem cells provide a potential source of cells for the regeneration of tissues, medical treatment, and research. In the past, embryos were the only source of stem cells with the capacity to differentiate into adult tissues, but because of ethical concerns about creating and using human embryos for harvesting stem cells, researchers have long sought the ability to induce adult somatic cells to dedifferentiate and revert to stem cells. Such cells are called **induced pluripotent stem cells (iPSCs)**. Researchers have now successfully created iPSCs by treating fibroblasts (fully differentiated human connective-tissue cells) in culture with a cocktail of transcription factors (**Figure 21.7**), although less than 1% of the cells that are treated in this way actually revert to iPSCs. Transcription factors that induce pluripotency cause extensive epigenetic reprogramming, altering the patterns of DNA methylation and histone modifications that accumulate with cell differentiation. Research has shown, however, that iPSCs retain a memory of their past and are not completely equivalent to embryonic stem cells (those derived from embryos). One study found that although the DNA methylation patterns of iPSCs differ greatly from those of differentiated somatic cells, the iPSCs retained some methylation marks of the somatic cells, and the methylation of iPSCs was not identical to that of

embryonic stem cells. Another study compared histone modifications of fibroblasts, iPSCs, and embryonic stem cells. The iPSCs and embryonic stem cells had fewer H3K27me3 and H3K9me3 marks than the fibroblasts did, but researchers also found significantly more of these marks on the iPSCs than on the embryonic stem cells.



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21.7 Differentiated adult cells can be reprogrammed to form induced pluripotent stem cells (iPSCs), which are capable of differentiating into many different types of cells.

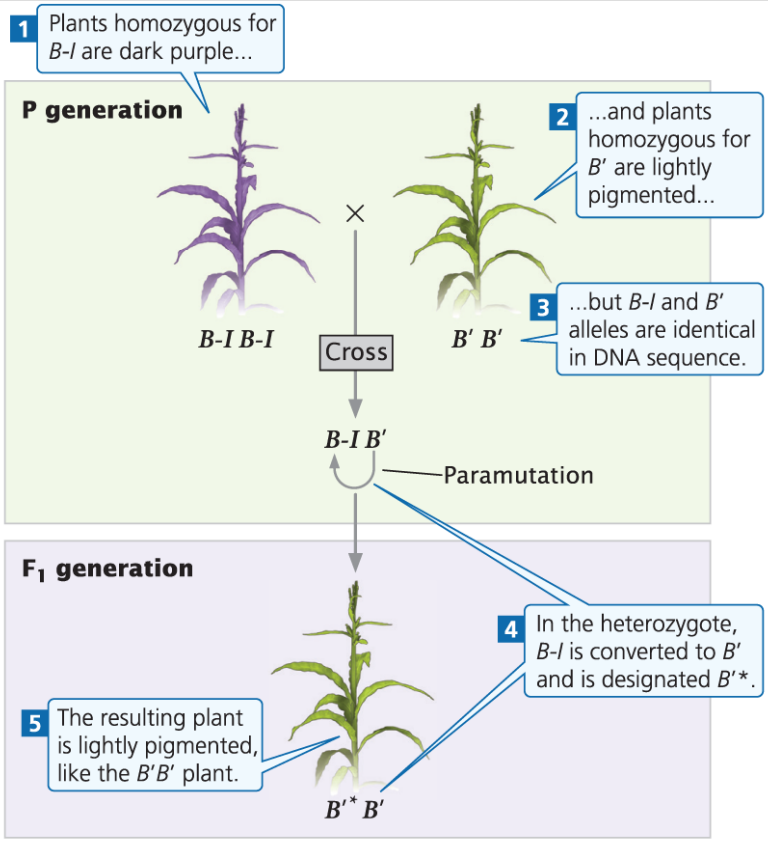
Description
The flowchart starts with a cluster of adult cells. Transcription factors are added into them which lead to i P S C's. A caption reads, "Upon exposure to transcription factors, a few adult cells undergo epigenetic reprogramming and become i P S C's, capable of differentiating into many cell types." The i P S C's then branch into three types of cells as follows: endoderm (the internal layer), mesoderm (the middle layer), and ectoderm (the external layer). Three pictures of endodermal cells are drawn: lung, thyroid, and pancreatic cells. 5 types of mesodermal cells are drawn: cardiac muscle, skeletal muscle, tubule cells of the kidney, red blood, and smooth muscle cells (in gut). 3 types of ectodermal cells are drawn: neuron, skin cells of epidermis, and pigment cells. A caption reads, "Epigenetic modifications during cell differentiation silence some genes and activate others in each cell type."

Transgenerational Effects Through Paramutation

Some epigenetic effects extend across generations, affecting future offspring. One type of transgenerational epigenetic effect is paramutation, defined as an interaction between two alleles that leads to a heritable change in the expression of one of the alleles. Surprisingly, paramutation produces these changes in phenotype without any alteration in the DNA base sequence of the converted allele. The phenomenon of paramutation has several important features. First, the newly established expression pattern of the

converted allele is transmitted to future generations, even when the allele that brought about the alteration is no longer present. Second, the altered allele is now able to convert other alleles to the new phenotype. And third, there are no associated DNA sequence changes in the altered alleles. A number of examples of paramutation have now been discovered in different organisms, and geneticists have begun to unravel the molecular mechanism of this curious phenomenon.

An example of paramutation occurs at the *b1* locus in corn, which helps determine the amount of purple anthocyanin the corn plant produces. The locus actually encodes a transcription factor that regulates genes involved in anthocyanin production. Plants homozygous for the *B-I* allele (*B-I B-I*) show high expression of the *b1* locus and are dark purple in color (**Figure 21.8**). Plants homozygous for the B' allele (B' B') show a lower expression of the *b1* locus and are lightly pigmented. However, the DNA sequences of the *B-I* and B' alleles are identical. Genetically identical alleles such as these, which produce heritable differences in phenotypes through epigenetic processes, are referred to as **epialleles**.



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21.8 In paramutation at the *b1* locus in corn, a copy of the B' allele converts the *B-I* allele to B'^*, which has the same phenotype as B'. The *B-I B-I* genotype produces a pigmented plant, while the $B'\ B'$ and $B'\ B'^*$ genotypes are lightly pigmented.

Description

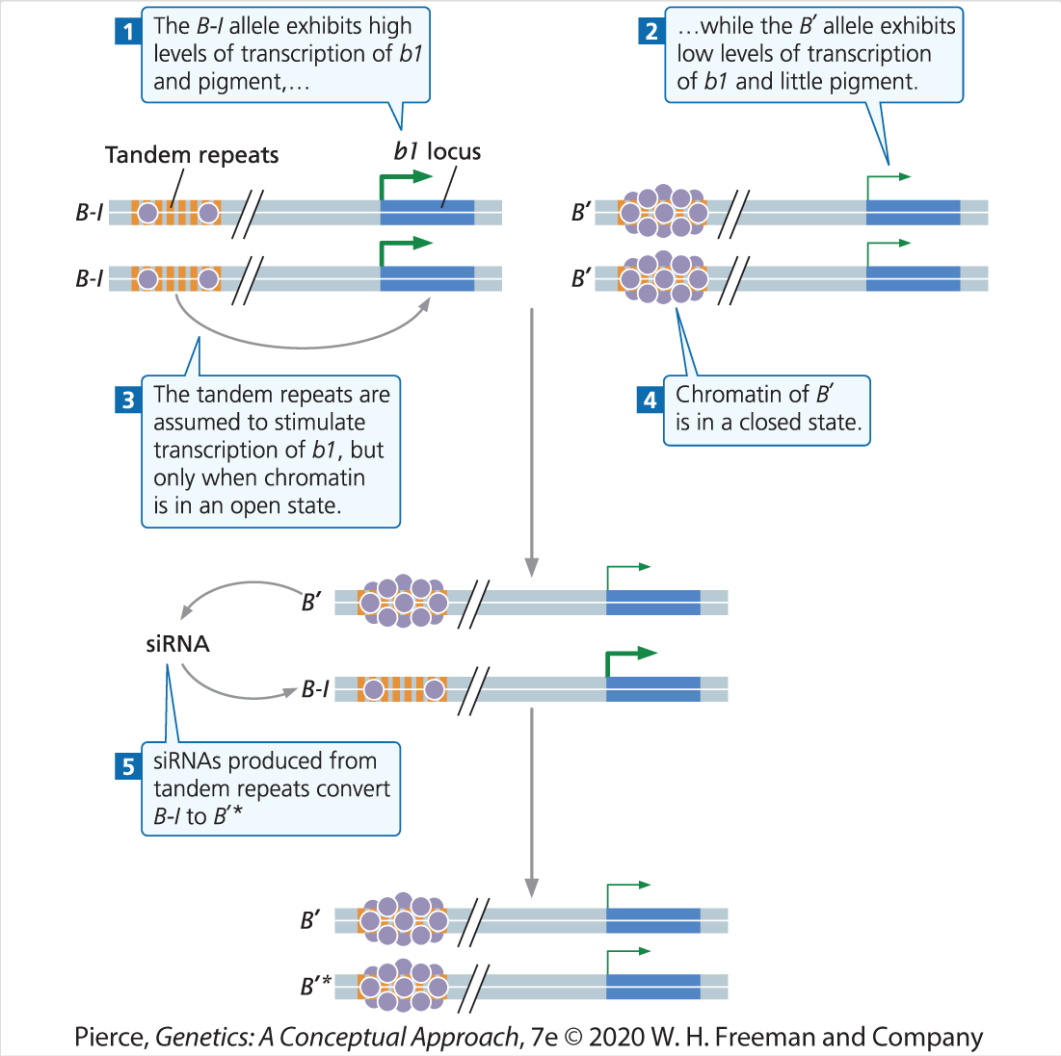
The illustration starts with a cross between two corn plants with genotypes B-1 B-1 and B prime B prime, respectively in the P generation. A caption reads, “Plants homozygous for B-1 are dark purple and plants homozygous for B prime are lightly pigmented but B-1 and B prime alleles are identical in D N A sequence.” The cross leads to formation of B-1 B prime, in which paramutation occurs. An arrow from B prime points towards B. In F subscript 1 generation, this B-1 B prime leads to a plant with genotype B prime asterisk B prime. A caption pointing towards paramutation after the cross and the genotype of the F subscript 1 generation offspring reads, “In the heterozygote, B-1 is converted to B prime

and is designated B prime asterisk." Another caption pointing to the plant in F subscript 1 generation reads, "The resulting plant is lightly pigmented, like the B prime B prime plant."

In plants that are heterozygous *B-I* B', the *B-I* allele is converted to B', with the result that the heterozygous plants are lightly pigmented (see Figure 21.8), just like the B' B' homozygotes. The newly converted allele is usually designated B'^{*}. Importantly, there is no functional difference between B' and B'^{*}; the B'^{*} allele is now fully capable of converting other *B-I* alleles into B'^{*} alleles in subsequent generations.

Research has demonstrated that one of the features required for paramutation at the *b1* locus is the presence of a series of seven tandem repeats of an 853-bp sequence located approximately 100,000 base pairs upstream of the coding sequence for the *b1* locus (**Figure 21.9**). The repeats do not encode any protein. Both the *B-I* and B' alleles have these tandem repeats, but the chromatin structure of the two alleles differs: the *B-I* allele has more open chromatin. The tandem repeats are required for high expression of the *B-I* allele and high pigment production. It has been suggested that the repeats act like an enhancer (see Chapter 17), stimulating transcription at the *b1* locus, but only when the chromatin surrounding the repeats is in an open configuration, as it is in the *B-I* allele. The more closed configuration in the B' allele may prevent the repeats from interacting with the promoter of *b1* and thus

stimulating transcription. How the repeats might interact with the B' allele is not known.



21.9 Paramutation at the *b1* locus in corn requires the presence of seven tandem repeats upstream of the *b1* locus.

Description

The illustration starts with two chromosomes. The chromosome on the left has tandem repeats at one end and a blue region labeled b 1 locus at the other. This chromosome is labeled B-1 on each strand. The chromosome on the right shows a cluster of small circles at one end and a blue region at the other. A caption reads, “The B-1 allele exhibits high

levels of transcription of b 1 and pigment, while the B’ allele exhibits low levels of transcription of b 1 and little pigment.” A caption pointing towards the tandem repeats on left chromosome reads, “The tandem repeats are assumed to stimulate transcription of b 1, but only when chromatin is in an open state.” A caption pointing towards a cluster of small circles on the right chromosome reads, “Chromatin of B prime is in a closed state.” The two chromosomes lead to a single chromosome with two strands. Its upper strand is similar to the chromosome on the right labeled B prime and its lower strand is similar to the chromosome on left labeled B-1. An arrow from B prime leads to s i R N A, further leading to B-1. A caption pointing towards s i R N A reads, “s i R N A’s produced from tandem repeats convert B-1 to B prime asterisk.” A downward arrow from the chromosome B prime B-1 leads to a chromosome in which both the strands have a cluster of small circles on one side and a blue region on the other. The upper strand is labeled B prime, while the lower strand is labeled B prime asterisk.

The different chromatin states of *B-I* and B' may explain their different levels of expression, but how does the B' allele convert the *B-I* allele to B'^{*}? Although the mechanism is not completely understood, recent research demonstrates that the communication between B' and *B-I* probably occurs through the action of small RNA molecules. The tandem repeats that are required for paramutation encode 25-nucleotide-long siRNAs (Figure 21.9). Some siRNAs are known to modify chromatin structure by directing DNA methylation to specific DNA sequences. Current evidence suggests that siRNA molecules convert *B-I* to B'^{*} and that this conversion involves a change in the chromatin states of the alleles. Research also shows that the transcription of the tandem repeats and the generation of siRNAs from them are necessary, but not sufficient, for paramutation, so additional factors must be involved.

Paramutation is not unique to plants. Several examples of paramutation have also been observed in mice, including paramutation at the *Kit* locus, which affects pigmentation of the feet and tail.

THINK-PAIR-SHARE Questions 3 and 4

CONCEPTS

Paramutation occurs when one allele creates a heritable alteration in another allele without any change in DNA sequence. Research suggests that paramutation in corn is mediated through small RNA molecules.

✔ CONCEPT CHECK 3

Which of the following is a characteristic of paramutation?

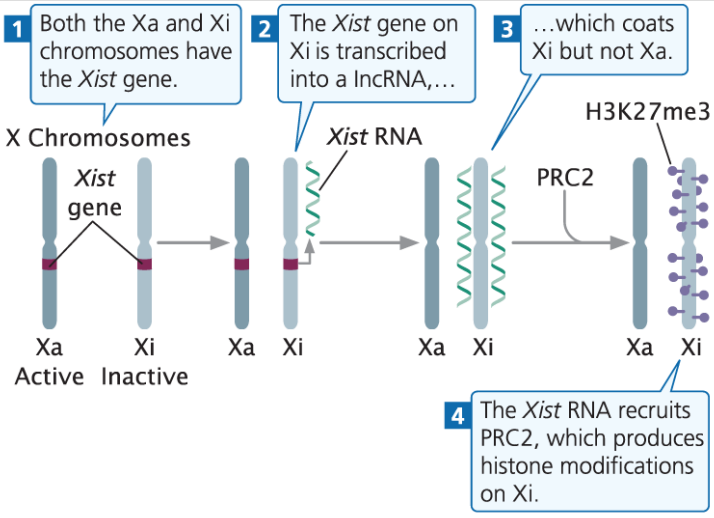
a. One allele is able to alter another allele when both are present in a heterozygote.
b. Altered alleles are passed on to future generations.
c. Altered alleles are capable of altering other alleles in future generations.
d. All of the above

X Inactivation

Early in the development of female mammals, one X chromosome in each cell is randomly inactivated to provide equal expression of X-linked genes in males and females (dosage compensation; see

Section 4.3). Through this process, termed X inactivation, many genes on the inactivated X chromosome are permanently silenced and are not transcribed. Once a particular X chromosome is inactivated in a cell, that same X chromosome remains inactivated when the DNA is replicated, and the inactivation mark is passed on to daughter cells through mitosis. This phenomenon is responsible for the patchy distribution of black and orange pigment seen in tortoiseshell cats (see Figure 4.19). X inactivation is a type of epigenetic effect because it results in a stable change in gene expression that is passed on to other cells.

A great deal of research has demonstrated that the particular X chromosome inactivated within a cell is controlled by a particular segment of the X chromosome called the **X-inactivation center**, which is 100,000 to 500,000 bp in length. Inactivation is initiated at the X-inactivation center and then spreads to the remainder of the inactivated X chromosome. Examination of the X-inactivation center led to the discovery of several genes that play a role in inactivating all but one X chromosome in each female cell and keeping the other X chromosome active (**Figure 21.10**).



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21.10 In X inactivation, the *Xist* gene on the inactive X produces a long noncoding RNA that coats the inactive X chromosome and suppresses transcription.

Description

The illustration starts with two X-chromosomes. One is labeled X a active and the other is labeled X i inactive. Both the chromosomes show Xist gene on the lower arm. A caption reads, “Both the X a and X i chromosomes have the Xist gene.” From the Xist gene on X i chromosome, Xist R N A is produced. The transcribed R N A then covers the X i chromosome on both sides. A caption reads, “The Xist gene on X i is transcribed into a lnc R N A, which coats X i but not X a.” The chromosomes are then subjected to P R C 2, which causes the H 3 K 27 me 3 to attach on the X i chromosome. A caption reads, “The Xist R N A recruits P R C 2, which produces histone modifications on X i.”

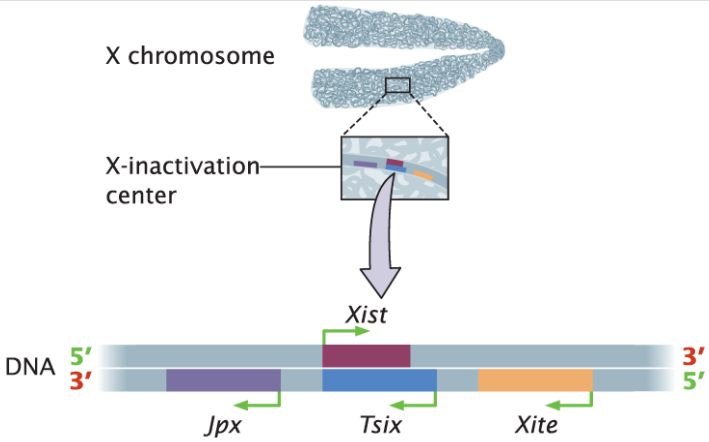
The key player in X inactivation is a gene called *Xist* (for *X*-*i*nactive *s*pecific *t*ranscript), which encodes a long noncoding RNA (lncRNA) that is 17,000 bp in length (Figure 21.10). As its name implies, this RNA molecule does not encode a protein. Instead, *Xist* lncRNA coats the X chromosome from which it was transcribed. *Xist* lncRNA then

attracts Polycomb repressive complex 2 (PRC2) and, later, Polycomb repressive complex 1 (PRC1). These proteins produce epigenetic marks, such as H3K27me3, and other histone modifications that repress transcription. Eventually, many CpG dinucleotides are methylated, leading to the permanent silencing of the inactivated X chromosome.

In mice, there are two separate inactivation events. Soon after fertilization, when the embryo reaches the eight-cell stage, the X chromosome from the male parent is inactivated, while the maternal X chromosome remains active. This event is called imprinted X-chromosome inactivation. In the developing embryo, the paternal X chromosome is then reactivated during blastocyst maturation. Inactivation occurs again in early development, but now which X is inactivated is random: the X from the male parent and the X from the female parent are equally likely to be inactivated. From this point on, whichever X is inactivated remains silenced through subsequent cell divisions. However, some genes on the inactivated X chromosome escape inactivation and continue to be transcribed. How these genes escape X inactivation is not known. Interestingly, in marsupial mammals, the paternal X chromosome is the copy that remains permanently silenced in all cells.

As mentioned, X inactivation is brought about by the transcription of the *Xist* gene on the inactive X chromosome; this transcription produces *Xist* lncRNA, which coats the inactive X chromosome and leads to changes in chromatin structure that silence transcription.

But what happens on the active X chromosome? Why isn't it coated by *Xist* RNA and silenced? Although all the details of this process are not completely understood, researchers have determined that there are several additional genes in the X-inactivation center that encode other lncRNAs (**Figure 21.11**). These lncRNAs help bring about the inactivation of the inactive X, while also not silencing the active X (see Figure 21.10). One of these genes is the *Tsix* gene, which is transcribed on the active X chromosome. *Tsix* is antisense to *Xist*, which means that it overlaps with the *Xist* gene and is transcribed from the opposite strand (see Figure 21.11), producing a *Tsix* lncRNA that is complementary to *Xist* lncRNA. Through several mechanisms, *Tsix* represses the expression of *Xist* on the active X chromosome. Another major player is a gene called *Jpx*, which encodes a lncRNA that stimulates the transcription of *Xist* on the inactive X chromosome. Thus, *Xist* is controlled by two parallel switches with opposite effects: (1) *Jpx* stimulates *Xist* expression on the inactive X chromosome, causing *Xist* to be transcribed and leading to X inactivation; and (2) *Tsix* represses *Xist* on the active X chromosome, causing *Xist* not to be transcribed on that chromosome and preventing inactivation. Several other genes are also involved. A gene called *Xite* encodes a lncRNA that sustains *Tsix* expression on the active X chromosome. The major genes involved in the process of X inactivation are summarized in **Table 21.2**.



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21.11 Several genes within the X-inactivation center interact to bring about the inactivation of one X chromosome while keeping the other X chromosome active. Shown is the mouse X chromosome, X-inactivation center, and the genes involved in X-inactivation.

Description
The illustration starts with a horizontally placed V-shaped X-chromosome. It shows an X-inactivation center in between. The magnified image of the X-inactivation center shows a double-stranded D N A with the following genes: First strand, 5 prime-Xist gene at thecenter-3 prime; second strand, 3 prime-J p x gene (left), T six gene (center), and Xite gene (right)-5 prime.

TABLE 21.2 Major genes involved in X inactivation

Gene	Encodes	Action of Gene
Xist	lncRNA	Coats inactive X chromosome and leads to the silencing of transcription of many genes on the inactive X
Tsix	lncRNA	Inhibits transcription of *Xist* on the

		active X chromosome
Jpx	lncRNA	Stimulates transcription of *Xist* on the inactive X chromosome
Xite	lncRNA	Sustains *Tsix* expression on the active X chromosome, which inhibits *Xist* and maintains transcription of genes on the active X chromosome

This complex process ensures that in each female cell, one X chromosome is inactivated and one remains active. Scientists have long recognized that X inactivation also involves some type of mechanism that is capable of counting X chromosomes, because all but one X chromosome in each cell is inactivated. Thus, the single X in the cells of an XY male remains active (no X inactivation occurs), and two X chromosomes are inactivated in XXX females (see discussion of Barr bodies in Section 4.3). The nature of this counting mechanism is not yet well understood. Learn more about the mechanism of X inactivation in (A) **Animation 21.1**. **› TRY PROBLEM 29**

CONCEPTS

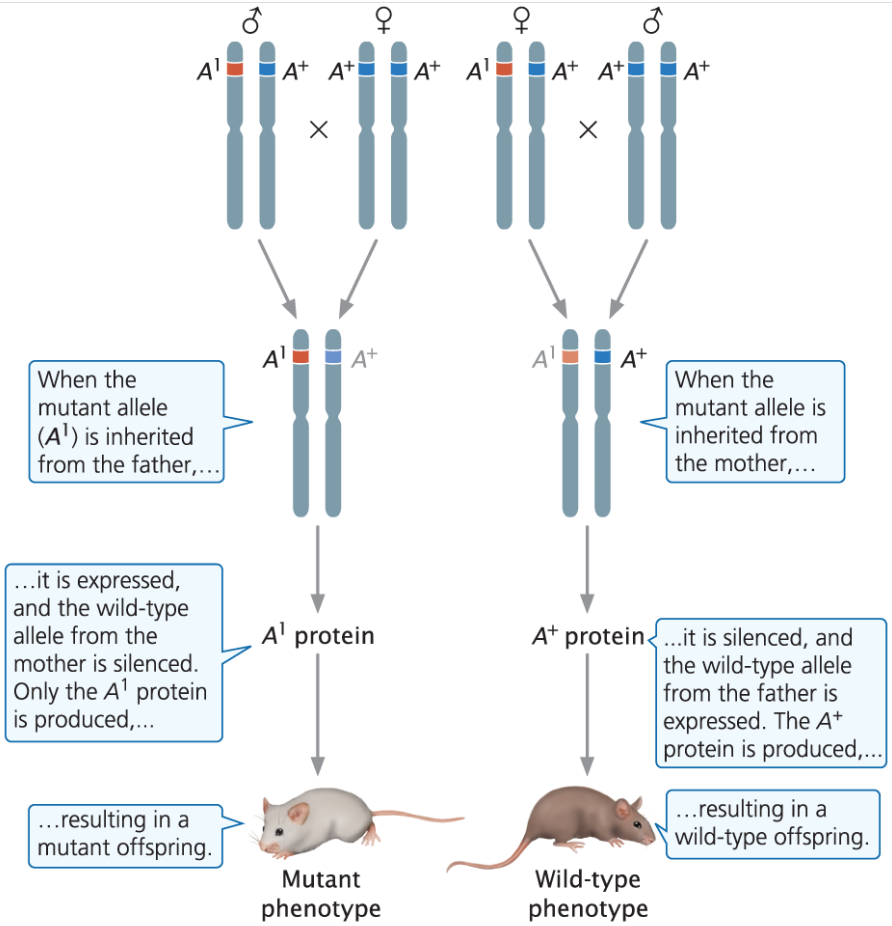
Epigenetic changes underlie X inactivation, in which one X chromosome in female cells is permanently silenced. X inactivation occurs through the action of several genes in the X-inactivation center that encode long noncoding RNAs. The products of these genes interact to ensure that one X chromosome is inactive and one remains active in each female cell.

✔ CONCEPT CHECK 4

What would be the effect of introducing siRNAs that degrade *Xist* RNA into a female cell?

Genomic Imprinting

Diploid organisms usually possess two alleles at each autosomal locus, with one allele inherited from the mother and one allele inherited from the father. For most genes, both alleles are expressed, and the effect of a particular allele on the phenotype is independent of which parent transmitted that allele to the offspring. However, for some genes, the sex of the parent that contributed the allele influences how that allele is expressed—alleles inherited from the mother and from the father are not equivalent (**Figure 21.12**). This phenomenon, in which the sex of the parent that transmits the allele determines its expression, is termed genomic imprinting. For some imprinted genes, when the allele is inherited from the male parent, it is expressed, but when it is inherited from the female, it is silent; for other genes, when the allele is inherited from the female parent, it is expressed, but when it is inherited from the male, it is silent. As discussed in Section 5.3, genomic imprinting is thought to be due to different degrees of methylation of the alleles inherited from the two parents.



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21.12 In genomic imprinting, the expression of an allele depends on whether it is inherited from the male or the female parent. In this case, the A^1 allele is expressed only when inherited from the male parent, but in other cases, an allele is expressed only when inherited from the female parent.

Description

The illustration starts with two crosses. One cross is between a male A 1 A superscript positive chromosome and a female A superscript positive A superscript positive chromosome. The other cross is between the same chromosome but with opposite sex. Both the chromosomes produce A 1 A superscript positive chromosome. A caption pointing towards the chromosomes produced from first cross reads, “When the mutant allele (A 1) is inherited from the father, it is expressed, and the wild-type allele from the mother is silenced. Only the A 1 protein is resulting in a mutant offspring.” The chromosomes produced from first cross leads to A superscript 1 protein which produces

a white mouse with mutant phenotype. A caption pointing towards the chromosomes produced from second cross reads, “When the mutant allele is inherited from the mother, it is silenced, and the wild-type allele from the father is expressed. The A superscript positive protein is produced, resulting in a wild-type offspring.” The chromosomes produced from second cross leads to A superscript positive protein which produces a black mouse with wild-type phenotype.

An interesting example of genomic imprinting involves crosses between a horse and a donkey. A cross between a male donkey and a female horse produces a mule, but a cross between a female donkey and a male horse produces a hinny. Mules and hinnies differ in appearance, physiology, and behavior; for example, hinnies are smaller than mules, have shorter ears, and have stronger legs. Because both mules and hinnies have the same genetic makeup (half donkey and half horse) and differ only in which sex conveys the horse and donkey genes, their differences are thought to be due to genomic imprinting. Studies of RNA levels in mules and hinnies demonstrate that they do indeed exhibit differential expression of some genes.

Previous research had suggested that the number of imprinted genes was limited, but more recent research suggests that the number is much higher than those suggested limits. A study conducted by Christopher Gregg, at Harvard University, and his colleagues found that over 1300 genes in the mouse brain exhibited evidence of genomic imprinting. Many of these imprinted genes were not completely silenced; instead, there was biased expression, with one sex transmitting an allele that was more highly expressed than the

allele transmitted by the other sex. Gregg and his colleagues also found that imprinting was highly variable; some genes were imprinted only in certain tissues or at certain times of development.

Genomic imprinting has a number of interesting parallels to X inactivation. Most imprinted genes are located in clusters of 3–12 genes that occur in a discrete region of a particular chromosome. Each cluster contains genes that encode proteins as well as genes that produce noncoding RNA. In each of the well-studied examples, there is an imprinting control region that determines imprinting; deletion of this region destroys the ability to imprint. In addition, the imprinting control region exhibits differences in chromatin modifications between alleles inherited from the male and female parents. Each imprinting cluster contains genes for one or more lncRNAs, which play an important role in imprinting and are themselves imprinted. For example, the gene for insulin-like growth factor 2 (*Igf2*) in humans exhibits genomic imprinting; the *Igf2* allele transmitted from the male parent is expressed, while the *Igf2* allele transmitted from the female parent is silenced (see Figure 5.18). Several lncRNAs produced by other genes in the imprinting control region are required for silencing of the female *Igf2* allele in the fetus, although how they bring about the repression of transcription is not clear.

Many of the well-studied clusters of imprinted genes are associated with disorders that result from faulty imprinting. Beckwith-Wiedemann syndrome is one such disorder. Children with Beckwith-

Wiedemann syndrome exhibit excessive growth during fetal development and early childhood. They also have unusual embryonic malignant tumors. Beckwith-Wiedemann syndrome is associated with imprinting in a cluster of genes on chromosome 11, including the *Igf2* gene. Individuals with Beckwith-Wiedemann syndrome often have small deletions on chromosome 11 that interfere with the normal process of imprinting. For example, *Igf2* is normally expressed only when inherited from the father, but in some children with Beckwith-Wiedemann syndrome, deletions within the imprinting center lead to the expression of alleles from both parents. The result is that too much *Igf2* is produced, leading to excessive growth and cancer. Prader–Willi syndrome and Angelman syndrome are disorders that occur due to imprinting defects on chromosome 15 (see Section 5.3).

IMPRINTING AND GENETIC CONFLICT

Many imprinted genes affect early embryonic and fetal growth. One possible explanation for genomic imprinting is the **genetic conflict hypothesis**, which suggests that there are different and conflicting evolutionary pressures acting on maternal and paternal alleles for genes (such as *Igf2*) that affect fetal growth. From an evolutionary standpoint, paternal alleles that maximize the size of the offspring are favored because birth weight is strongly associated with infant survival and adult health. Thus, it is to the advantage of the male parent to pass on alleles that promote maximum fetal growth in his

offspring. In contrast, maternal alleles that cause more limited fetal growth are favored because committing too many nutrients to any one fetus may limit a mother's ability to reproduce in the future, and giving birth to very large babies is also difficult and risky. The genetic conflict hypothesis predicts that genomic imprinting will evolve such that paternal copies of genes that increase fetal growth will be maximally expressed, whereas maternal copies of the same genes will be less actively expressed or even silent. Indeed, *Igf2* follows this pattern: the paternal allele is active and promotes growth; the maternal allele is silent and does not contribute to growth. Recent findings demonstrate that the paternal copy of *Igf2* promotes fetal growth by directing maternal nutrients to the fetus through the placenta.

THINK-PAIR-SHARE Question 5

CONCEPTS

Genomic imprinting is caused by epigenetic differences in the alleles inherited from male and female parents. The genetic conflict hypothesis suggests that imprinting evolves because of conflicting evolutionary pressures acting on maternal and paternal alleles.

✔ CONCEPT CHECK 5

Which of the following is true of genomic imprinting?

a. The sex of the parent that transmits an allele affects the expression of the allele in the offspring.

b. The sex of the offspring affects the expression of an allele inherited from one of the parents.
c. The sex of the parent affects how an allele is transmitted to the offspring.
d. The sex of the offspring affects which allele is inherited from the parent.

Epigenetic Effects in Monozygotic Twins

Monozygotic (identical) twins develop from a single egg fertilized by a single sperm that divides and gives rise to two zygotes (see Section 6.3). Thus, monozygotic twins are genetically identical, in the sense that they possess identical DNA sequences, but they often differ somewhat in appearance, health, and behavior. The nature of these differences in the phenotypes of identical twins is not well understood, but evidence suggests that at least some of these differences may be due to epigenetic changes. In one study, Mario Fraga, at the Spanish National Cancer Center, and his colleagues examined 80 pairs of identical twins and compared the degree and location of their DNA methylation and histone acetylation. They found that DNA methylation and histone acetylation in identical twin pairs were similar early in life, but that older twin pairs had remarkable differences in their overall content and distribution of DNA methylation and histone acetylation. Furthermore, these differences affected gene expression in the twins. This research suggests that identical twins do differ epigenetically and that

phenotypic differences between them may be caused by differential gene expression.

THINK-PAIR-SHARE Question 6

CONCEPTS

Phenotypic differences between genetically identical monozygotic twins may result from epigenetic effects.

✔ CONCEPT CHECK 6

To what extent would you expect to see differences in the DNA base sequences and epigenetic marks between pairs of monozygotic twins?

a. Similar differences in DNA base sequence and epigenetic marks
b. Greater differences in DNA base sequence than in epigenetic marks
c. Greater differences in epigenetic marks than in DNA base sequence
d. No differences in either DNA base sequence or epigenetic marks

21.4 Epigenetic Alterations Bring about Changes in Behavior, Reproduction, and Metabolism

Research studies demonstrate that epigenetic modifications are affected by and influence a number of important functions in life, including maternal behavior, response to stress, cognition, reproduction, and metabolism.

Behavioral Epigenetics

Research has shown that life experiences, especially those early in life, can have long-lasting effects on behavior, in some cases into future generations. Increasingly, researchers are finding that these long-term effects are mediated through epigenetic processes. The number of studies that convincingly demonstrate that life experience alters chromatin structure is currently small (and some are still controversial), but a number of researchers are actively looking for the epigenetic effects of experience and their long-term effects on chromatin structure and behavior.

EPIGENETIC CHANGES INDUCED BY MATERNAL BEHAVIOR

A fascinating example of behavioral epigenetics is seen in the long-lasting effects of maternal behavior in rats. A mother rat licks and grooms her offspring (Figure 21.13), usually while she arches her back and nurses them. The offspring of mothers who display more licking and grooming behavior are less fearful as adults and show reduced hormonal responses to stress compared with the offspring of mothers who lick and groom less. These long-lasting differences in the offspring are not due to genetic differences inherited from their mothers—at least not genetic differences in the base sequences of their DNA. Offspring exposed to more licking and grooming develop a different pattern of DNA methylation than do offspring exposed to less licking and grooming. These differences in DNA methylation affect the acetylation pattern of histone proteins. The altered acetylation pattern persists into adulthood and alters the expression of the glucocorticoid receptor gene, which plays a role in hormonal responses to stress. The expression of other stress-response genes is also affected.

Eric Isselee/Shutterstock.

21.13 Young rats exposed to more licking and grooming from their mothers develop different patterns of DNA methylation, which alters the expression of stress-response genes and makes them less fearful as adults, compared with the offspring of mothers who lick and groom less.

To demonstrate the effect of altered chromatin structure on the stress responses of the offspring, researchers infused the brains of young rats with a deacetylase inhibitor, which prevents the removal of acetyl groups from histone proteins. After the infusion of the deacetylase inhibitor, differences in DNA methylation and histone acetylation associated with maternal grooming behavior disappeared, as did the difference in responses to fear and stress in the young rats when they reached adulthood. This study demonstrates that the mother rat's licking and grooming behavior brings about epigenetic changes in the offspring's chromatin, which causes long-lasting differences in their behavior.› **TRY** PROBLEM 31

EPIGENETIC EFFECTS OF EARLY STRESS

Numerous studies have demonstrated that stress during childhood and adolescence produces a number of adverse effects that persist into adult life. For example, childhood abuse increases the probability that the child will experience depression, anxiety, and suicide as an adult. In one study, researchers examined the brains of 24 people who had committed suicide, half of whom had experienced childhood abuse. They found that those who had experienced childhood abuse had a greater degree of methylation of the glucocorticoid receptor gene, a gene involved in the stress response, than those who had not. Although the number of brains studied was small, the study suggests that early childhood stress

can indeed cause epigenetic modifications to chromatin structure in humans.

Other studies have demonstrated that gene expression is affected by early life experience. For example, researchers found that children who grew up in a lower socioeconomic environment before the age of 5 showed the altered expression of over 100 genes related to immune function as adults.

Research in mice suggests that the epigenetic effects of stress may be mediated by small RNA molecules. In these studies, male mice were subjected to chronic stresses, such as restraint, the odor of a predator, or noise. After they were exposed to the stress, the males were bred to females who had experienced no stress. Their offspring displayed a blunted hormonal response when stressed by restraint, a response called reduced hypothalamic–pituitary–adrenal (HPA) stress axis reactivity. Furthermore, these offspring exhibited an altered expression of the genes involved in the stress response.

The researchers found increased levels of nine miRNAs in the stressed fathers' sperm, which they believed conveyed the blunted hormonal response to the offspring. To test this hypothesis, they injected copies of these specific miRNAs into mouse embryos, whose parents had not been exposed to stressful conditions, and implanted the embryos into surrogate mothers. When these mice grew up, they exhibited the same blunted hormonal response as the

offspring of the stressed fathers, even though their parents had never been exposed to stressful conditions. Appropriate controls were included to ensure that the observed effect was not simply due to the injection procedure. The results demonstrated that miRNAs are involved in the epigenetic transmission of the altered hormonal response across generations. Using RNA sequencing to examine levels of transcription (see Section 20.2), the researchers also showed that the miRNAs brought about changes in gene expression associated with the altered stress response. Other studies have found that fragments of tRNAs passed through sperm similarly bring about epigenetic effects of diet that are passed from male mice to their offspring.

EPIGENETICS IN COGNITION

A number of research studies have shown that abnormalities in DNA methylation are associated with disorders of development and intellectual ability in humans. These findings prompted researchers to look for the effects of chromatin structure on learning, memory, and cognitive ability in mice and rats. One study found that training mice to avoid an aversive stimulus reduced DNA methylation of the *Bdnf* gene, which encodes a growth factor that stimulates the growth of connections between neurons. When demethylated, the *Bdnf* gene was more active. When researchers injected a drug that inhibits demethylation into the brains of the trained mice, activity

of the *Bdnf* gene was decreased, and the mice's memories of where the adverse stimulus occurred also decreased.

Another study found that a drug that promotes the acetylation of histone proteins improved learning and memory in mice that have a disorder similar to Alzheimer disease. Recall that the acetylation of histones alters chromatin structure by loosening the association of DNA with histone proteins and stimulates the transcription of many genes. Other studies have found that histone acetylation decreases with age in mice, resulting in diminished expression of genes related to learning and memory. When researchers injected mice with a drug that is an inhibitor of deacetylase activity, the acetylation of histones increased, the transcription of genes involved in memory increased, and the memories of the mice improved. These studies suggest that changes in chromatin structure may be involved in memory and learning.

THINK-PAIR-SHARE Question 7

CONCEPTS

Studies are providing evidence that early life experiences can produce epigenetic changes that have long-lasting effects on behavior.

Epigenetic Effects of Environmental Chemicals

Because some chemicals are capable of modifying chromatin structure, researchers have looked for long-term effects of environmental toxicants on chromatin structure and epigenetic traits.

There has been much recent interest in chemicals called endocrine disruptors, which mimic or interfere with natural hormones. Endocrine disruptors are capable of interfering with processes regulated by natural hormones, such as sexual development and reproduction. One of these endocrine disruptors is vinclozolin, a common fungicide used to control fungal diseases in vegetables and fruits—particularly wine grapes—and to treat turf on golf courses. Vinclozolin acts as an antagonist at the androgen receptor; that is, vinclozolin and its metabolites mimic testosterone and bind to the androgen receptor, preventing testosterone from binding. But vinclozolin and its metabolites do not properly activate the receptor, and in this way, vinclozolin inhibits the action of androgens and prevents sperm production.

In one study, researchers found that the exposure of embryonic male rats to vinclozolin led to reduced sperm production—not only in the treated animals when they reached puberty but also in several subsequent generations. Increased DNA methylation was

seen in the sperm of the males that were exposed to vinclozolin, and these patterns of methylation were inherited by the offspring of those males. This study and others have raised concerns that through epigenetic changes, environmental exposure to some chemicals might have effects on the health of future generations. ›

TRY PROBLEM 32

THINK-PAIR-SHARE Question 8

CONCEPTS

Through epigenetic changes, exposure to certain chemicals in the environment may have influences that extend to later generations.

Epigenetic Effects on Metabolism

In the introduction to this chapter, we saw that nutrition during prenatal development can have effects on health in later life. These types of epidemiological studies on humans are supported by laboratory studies of mice and rats. In one study, researchers fed inbred male mice either a normal (control) diet or a diet low in protein. They then bred mice in both groups to control females that were fed a normal diet. The males were then separated from the females and never had any contact with their offspring; their only

contribution to the offspring was a set of paternal genes transferred through the sperm.

Researchers raised the offspring and then examined their cholesterol levels. The offspring of males fed a low-protein diet exhibited increased expression of genes involved in lipid and cholesterol metabolism, and a corresponding decrease in levels of cholesterol, compared with the offspring of males fed a normal diet. The researchers also observed numerous differences in DNA methylation in the offspring of the two groups of fathers, although no differences could be found in the methylation patterns of the sperm of the two groups of fathers. These results suggest that epigenetic changes altered the cholesterol metabolism of the offspring, although how the differences in methylation were transmitted from father to offspring was unclear.

In another study, researchers fed male rats a high-fat diet and, not surprisingly, the rats gained weight. The researchers then bred these males to females that had been fed a normal diet. The offspring were also fed a normal diet. The daughters of the male rats on the high-fat diet had normal weight, but as adults they developed a diabetes-like condition of impaired glucose tolerance and insulin secretion. The researchers observed that in the insulin-secreting pancreatic islet cells of the daughters, the expression of 642 genes involved in insulin secretion and glucose tolerance was altered, demonstrating that a father's diet affected gene expression in his daughters.

21.5 The Epigenome

In 2003, researchers declared that essentially the entire human genome had been sequenced. This monumental achievement provided a wealth of information about how genetic information is encoded within the genome. Yet the DNA base sequence is only a partial record of heritable information. As we have discussed, additional epigenetic information is contained within chromatin structure—information that is heritable and that affects how the DNA base sequence is expressed. The overall pattern of chromatin modifications in a genome has been termed the **epigenome**. Researchers have now described epigenetic modifications across the genome.

GENOME-WIDE EPIGENETIC MARKS

Using the techniques we described earlier (methylation-sensitive endonucleases, bisulfite sequencing, and chromatin immunoprecipitation), geneticists have compared the epigenomes of different types of cells. For example, researchers determined the distribution of 5-methylcytosine across the entire genome in two cell types: (1) an undifferentiated human stem cell and (2) a fibroblast. The researchers found widespread differences in the methylation patterns of these cells. The finding that patterns of DNA methylation vary among cell types supports the idea that

cytosine methylation provides the means by which cell types stably maintain their differences during development. Other researchers have compared the epigenomes of cancer cells and normal cells, and they have observed distinct epigenetic marks associated with cancer. Some anticancer therapies are being developed that focus on these epigenetic modifications.

Similarly, researchers have mapped the genomic locations of histone modifications in different cell types. These studies have detected specific histone modifications associated with promoters and enhancers of active genes. In one study, researchers mapped nine different epigenetic marks in nine different types of human cells. They were able to determine how the marks varied across cell types and to compare the marks associated with active and repressed genes. Because specific epigenetic marks are often associated with regulatory elements such as promoters and enhancers, researchers have used the presence of these marks to map the locations of these regulatory elements throughout the genome.

The ability to map epigenetic modifications has allowed researchers to look for associations between the presence of epigenetic changes at specific locations and the presence of individual phenotypes. For example, one might look to see whether epigenetic modifications at specific loci are associated with diabetes, with the goal of identifying epigenetic changes that contribute to the disease. These types of studies are called **epigenome-wide association studies**

(EWAS) and are analogous to genome-wide association studies (GWAS, discussed in Chapters 7 and 20). One problem with EWAS research is that the epigenome potentially changes over an individual's lifetime and may be influenced by environmental factors. Thus, it is difficult to know whether epigenetic changes associated with a phenotype cause the phenotype or are caused *by* the phenotype, an effect known as reverse causation. For example, one might detect a statistical association between the presence of a disease (say, high blood pressure) and an epigenetic modification at a specific locus. This might indicate that the epigenetic modification contributes to high blood pressure, but it is also possible that high blood pressure causes the epigenetic modification.

Recent advances have made it possible to detect and measure genome-wide epigenetic modifications within single cells, similar to the ability to sequence DNA and RNA in single cells (see Chapter 20). With single-cell epigenomics, researchers study how different cell types vary in the presence of specific epigenetic changes, such as DNA methylation and histone modification. Single-cell epigenomics is being coupled with single-cell DNA sequencing and single-cell RNA sequencing to create triple-omics, which provides information about DNA variation, the alteration of chromatin structure, and gene expression.

ANCIENT EPIGENOMES

Neanderthals and Denisovans are two species of hominid that went extinct some 30,000 to 40,000 years ago (see the introduction to Chapter 10). In recent years, DNA has been isolated from their bones and sequenced, providing a detailed view of their genomes. Research now provides complementary information about their epigenomes.

Traditional methods of detecting DNA methylation, such as bisulfite sequencing, do not work well with ancient DNA because it is often present only in small amounts and is degraded. However, other methods, computational in nature, make it possible to identify regions of the DNA that were originally methylated. This is possible because, although DNA naturally decays after death, methylated and unmethylated cytosine bases do not decay in the same way. Unmethylated cytosine undergoes deamination to uracil (see Section 18.2), but methylated cytosine decays to thymine. These differences make it possible to determine the methylation status of the DNA prior to death. Before sequencing, uracil can be eliminated chemically, or a sequencing method that does not detect uracil can be used. Thus, those sites at which unmethylated cytosine has decayed to uracil are not read. Thymine, however, is read, so sites with methylated cytosine that decayed to thymine are read as thymine. This means that the ratio of thymine to cytosine bases that are read during sequencing will be higher in those regions of the DNA that were originally methylated than in regions that were not methylated.

By using the ratio of thymine to cytosine bases read during sequencing of Neanderthal and Denisovan DNA, scientists have determined the DNA methylation patterns of their genomes. The methylation patterns of over 99% of the genomes of these ancient hominids were similar to those observed in modern humans; this is not unexpected, given the close evolutionary relationships of Neanderthals, Denisovans, and *Homo sapiens*. However, there were a number of regions for which patterns of methylation differed among the species. One such region was in a group of five genes known as the *HOXD* cluster, which is an important regulator of limb development in mammals. These results suggest that this cluster of genes may have played an important role in the limb development of modern humans. Other differences in methylation were seen in enhancers and in transcription-factor binding sites. In addition, differential methylation in modern humans was seen more often in genes related to diseases, particularly neurological and psychiatric disorders such as autism and schizophrenia. This observation suggests that the epigenetic changes in gene expression that have evolved in modern humans might have increased the risk of some human diseases.

CONCEPTS

The epigenome is the complete set of chromatin modifications in a genome. Research reveals that the epigenomes vary among cell types and among ancient hominids.

Chapter 21 Review

CHAPTER SUMMARY

Section 21.1 Learning Objective: Be able to compare and contrast epigenetics and traditional inheritance.

- The term *epigenetics* refers to the mechanisms by which changes in phenotype are maintained in a cell, or passed to other cells or future generations, without a change in the base sequence of DNA.
- Many epigenetic phenotypes result from changes in chromatin structure.
- Epigenetic changes are stable but can be affected by environmental factors.

Section 21.2 Learning Objective: Be able to describe types of molecular changes that lead to epigenetic effects.

- Three molecular mechanisms underlie many epigenetic phenotypes: (1) changes in patterns of DNA methylation; (2) chemical modifications of histone proteins; and (3) RNA molecules that affect chromatin structure and gene expression.
- Some epigenetic effects result from DNA methylation, in which cytosine bases are methylated to form 5-methylcytosine.

Methylation often occurs at CpG dinucleotides. The presence of 5-methylcytosine is associated with the repression of transcription.

- DNA regions with many CpG dinucleotides are referred to as CpG islands. Methylation of CpG islands near a gene often leads to the repression of transcription.
- DNA methylation is maintained across cell divisions by methyltransferase enzymes that recognize methylation of CpG dinucleotides on one strand of DNA and add methyl groups to the unmethylated cytosine bases on the other strand.
- DNA methylation inhibits transcription by inhibiting the binding of transcription factors and other proteins required for transcription to occur. DNA methylation also attracts proteins that repress transcription and histone deacetylase enzymes that alter chromatin structure.
- DNA methylation can be detected using methylation-sensitive restriction endonucleases and bisulfite sequencing.
- Modifications of histone proteins alter chromatin structure. Histone modifications may be maintained across cell divisions.
- Modifications of histone proteins can be detected with immunoprecipitation methods.
- RNA molecules bring about modifications of chromatin by a variety of processes.

Section 21.3 Learning Objective: Be able to describe epigenetic effects on development.

- Extensive epigenetic reprogramming occurs during cell differentiation, silencing some genes and activating others.

- Paramutation is a heritable alteration of one allele by another allele without any change in DNA sequence. Paramutation in corn is mediated through small RNA molecules.
- X inactivation occurs when one X chromosome in female cells is permanently silenced. Epigenetic changes bring about X inactivation and require the action of several genes that encode long noncoding RNAs.
- Genomic imprinting occurs when the expression of a gene depends on which parent transmitted the gene. It is caused by epigenetic changes in chromatin structure that are passed to offspring. The genetic conflict hypothesis suggests that imprinting evolves because of conflicting evolutionary pressures acting on maternal and paternal alleles.
- Phenotypic differences between genetically identical monozygotic twins may result from epigenetic effects.

Section 21.4 Learning Objective: Be able to explain how epigenetic alterations bring about changes in behavior, reproduction, and metabolism.

- Early life experiences can produce epigenetic changes that have long-lasting effects on behavior.
- Environmental chemicals may produce epigenetic effects that are passed to later generations.
- Epigenetic modifications have effects on metabolism that extend across generations.

Section 21.5 Learning Objective: Be able to define epigenome and describe some findings of epigenomics.

- The epigenome is the complete set of chromatin modifications in a genome.

WORKED PROBLEM

The *b1* locus encodes a transcription factor that stimulates the production of anthocyanin, a purple pigment in plants. What would be the effect of deleting the seven tandem repeats that are located 100,000 bp upstream of the *b1* locus in corn?

» Solution Strategy

What information is required in your answer to the problem?

The effect of deleting the repeats.

What information is provided to solve the problem?

- The *b1* locus encodes a transcription factor that stimulates anthocyanin production in plants.
- The seven tandem repeats are located upstream of the *b1* locus.

For help with this problem, review:

Transgenerational Effects Through Paramutation in Section 21.3.

» Solution Steps

The information provided in the text indicates that the *B-I B-I* plant normally shows high expression of the *b1* locus, produces anthocyanin, and is purple in color. The tandem repeats are required for high transcription of the *b1* locus, which encodes a transcription factor that stimulates the production of anthocyanin, a purple pigment. The tandem repeats act like an enhancer, stimulating the transcription of the *b1* locus in the *B-I B-I* genotype. Without the enhancer-like action of the repeats, *b1* will be transcribed at minimal levels and little anthocyanin will be produced. This will result in lightly pigmented *B-I B-I* plants, the same phenotype as is usually seen in plants with genotype B' B'.

> **Recall:** Enhancers stimulate transcription at genes that may be distant from the enhancer (see Chapter 17).

The tandem repeats also encode 25-nucleotide-long siRNAs, which are required for paramutation (conversion of *B-I* alleles into B'^{*} alleles). Deletion of the tandem repeats would thus eliminate the ability of B' alleles to carry out paramutation.

IMPORTANT TERMS

epigenetic mark

stem cell
pluripotency
induced pluripotent stem cell (iPSC)
paramutation
epiallele
X-inactivation center
genetic conflict hypothesis
epigenome
epigenome-wide association study

ANSWERS TO CONCEPT CHECKS

1. b
2. b
3. d
4. No *Xist* RNA would be present to coat the X chromosome, and X inactivation would not occur. Both X chromosomes would remain active.
5. a
6. c

Chapter 21 Assessment

COMPREHENSION QUESTIONS

Introduction

1. What is the thrifty phenotype hypothesis? How does it help to explain the long-term effects of famine on people conceived during the Dutch Hunger Winter?

Section 21.1

2. *What are the important characteristics of an epigenetic trait?

Section 21.2

3. What three molecular mechanisms alter chromatin structure and are responsible for many epigenetic phenotypes?

4. What is the major form of DNA methylation that is seen in eukaryotes? At what type of DNA sequence is DNA methylation usually found?

5. How does DNA methylation repress transcription?

6. Briefly explain how patterns of DNA methylation are transmitted across cell divisions.

7. What types of histone modifications are responsible for epigenetic phenotypes?

Section 21.3

8. What are induced pluripotent stem cells? How are they derived from adult somatic cells?

9. What is paramutation? What are the key features of this phenomenon?

10. What makes X inactivation an epigenetic phenotype?

11. Briefly describe the molecular processes that cause one X chromosome in each female cell to be active, and the other X chromosome to be inactivated.

12. Define *genomic imprinting*.

13. What is the genetic conflict hypothesis for the origin of genomic imprinting?

14. What evidence suggests that differences in monozygotic twins may be caused by epigenetic effects?

Section 21.4

15. What evidence suggests that cognition in mice is influenced by epigenetic changes?

16. Explain how vinclozolin acts as an endocrine disruptor.

17. Give an example of an epigenetic effect of diet on metabolism.

Section 21.5

18. What is the epigenome?

APPLICATION QUESTIONS AND PROBLEMS

Introduction

19. The introduction to this chapter describes the long-term effects of famine on people conceived during the Dutch Hunger Winter.
    a. What evidence suggests that these are epigenetic effects?
    b. What additional evidence would help demonstrate that these effects are due to epigenetic changes?

Section 21.1

20.

How do epigenetic traits differ from traditional genetic traits, such as the differences in the color and shape of peas that Mendel studied?

Section 21.2

21. What would be required to prove that a phenotype is caused by an epigenetic change?

22. Which honeybee in **Figure 21.4** (the worker or the queen) will have more copies of 5-methylcytosine in its DNA? Explain your answer.

23. *What would be the effect of deleting the *Dnmt3* gene in honeybees?

24. *Much of DNA methylation in eukaryotes occurs at CpG dinucleotides, but some individual cytosine nucleotides are also methylated to form 5-methylcytosine. Considering what you know about the process by which DNA methylation at CpG dinucleotides is maintained across cell division, do you think that methylation at individual C nucleotides would also be maintained by the same process? Explain your reasoning.

25. A DNA fragment with the following base sequence has some cytosine bases that are methylated (indicated by C*) and others that are unmethylated. To determine the locations of methylated and unmethylated cytosines, researchers sequenced this fragment both with and without treatment with sodium bisulfite. Give the sequence of bases that will be read with and without bisulfite treatment.

—ATCGC*GTTAC*GTTGC*GTCA—

26. A geneticist is interested in determining the locations of methylated cytosines within a fragment of DNA. She treats some copies of the fragment with sodium bisulfite and leaves some copies untreated. She then sequences the treated and untreated copies of the fragment and obtains the following results. Give the original sequence of the DNA fragment, and indicate the locations of methylated cytosines.

Sequence without treatment:	—AATTGCCCGATCGATTAAGCCA—
Sequence with treatment:	—AATTGTTTGATCGATTAAGCTA—

27. CONNECT After reviewing the description of ChIP-Seq in Chapter 17, explain how ChIP-Seq can be used to determine the genomic locations of a specific histone modification, such as H3K4me3.

Section 21.3

28. CONNECT A cross between the F_1 individual in **Figure 21.8** and a plant with genotype *B-I B-I* will produce progeny with what phenotype? See Chapter 3 for information on determining genotypes of progeny in a genetic cross.

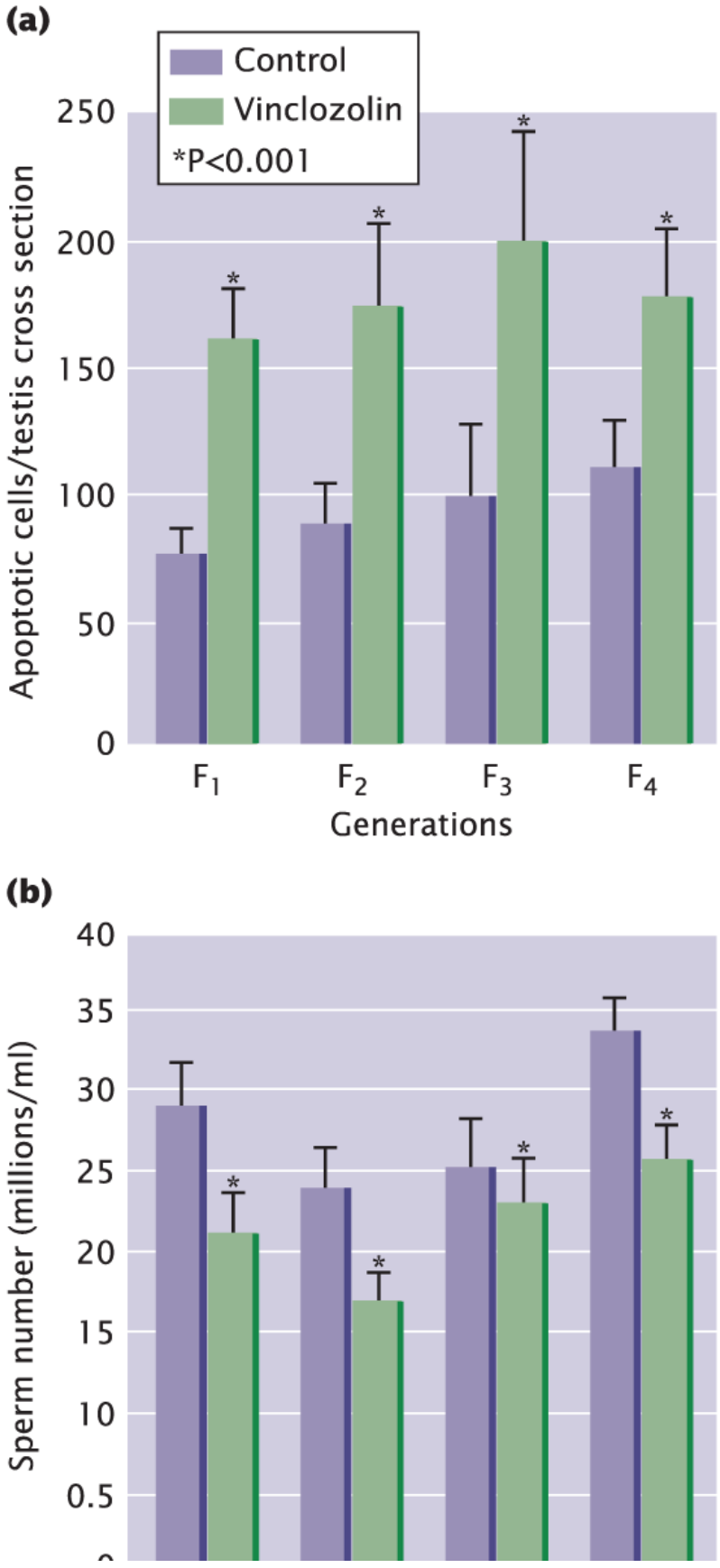
29. *How would the addition of siRNAs that eliminate the products of each of the following genes affect X inactivation?
    a. *Xist*
    b. *Jpx*

30. CONNECT Beckwith-Wiedemann syndrome is a genetic condition in which infants are born larger than normal and have other medical problems associated with excessive growth during fetal development. About 20% of the cases of Beckwith-Wiedemann syndrome are caused by uniparental disomy (see Chapter 8), in which both copies of a homologous pair of chromosomes come from the same parent. The children with Beckwith-Wiedemann syndrome have uniparental disomy for chromosome 11, a chromosome that contains several genes known to affect fetal growth. Using the genetic conflict hypothesis, predict whether these children have two copies of chromosome 11 from their father or two copies of chromosome 11 from their mother. Explain your answer.

Section 21.4

31. *A scientist does an experiment in which she removes the offspring of rats from their mother at birth and has her genetics students feed and rear the offspring. Assuming that the students do not lick and groom the baby rats as the mother rats normally would, what long-term behavioral and epigenetic effects would you expect to see in the rats when they grow up?

32. *Pregnant female rats were exposed to a daily dose of 100 or 200 mg/kg of vinclozolin, a fungicide commonly used in the wine industry (M. D. Anway et al. 2005. *Science* 308:1466–1469). The F_1 offspring of the exposed female rats were interbred, producing F_2, F_3, and F_4 rats. None of the F_2, F_3, or F_4 rats were exposed to vinclozolin. Testes from the F_1–F_4 male rats were examined and compared with those of control rats descended from females that had not been exposed to vinclozolin. The effects detailed in the accompanying graphs were seen in more than 90% of the F_1–F_4 male offspring. Furthermore, 8% of the F_1–F_4 males descended from vinclozolin-exposed females developed complete infertility, compared with 0% of the F_1–F_4 males descended from control females. Molecular analysis of the testes demonstrated that DNA methylation patterns differed between offspring of vinclozolin-exposed females and offspring of control females. Provide an explanation for the transgenerational effects of vinclozolin on male fertility.


(a)
Control
Vinclozolin
*P<0.001
Apoptotic cells/testis cross section
250
200
150
100
50
0
F1
F2
F3
F4
Generations
(b)
Sperm number (millions/ml)
40
35
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20
15
10
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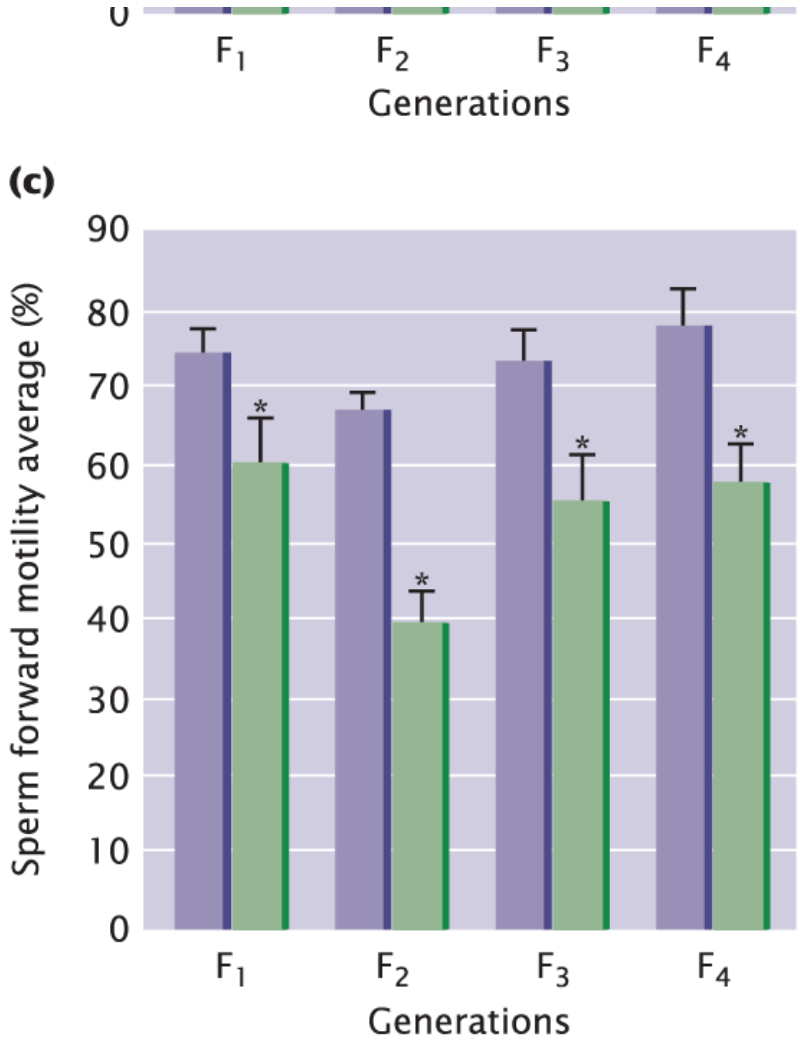

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[Data from M. D. Anway et al. 2005. *Science* 308:1466–1469.]

Description

All the graphs show data for control and vinclozolin. The horizontal-axis represents generations F 1 through F 4 in all the three graphs. The vertical-axis of the first graph is labeled apoptotic cells per testis cross section ranging from 0 to 250 with increments of 50. Approximate data from the first graph are as follows:

F 1: Control, 75; Vinclozolin, 160

F 2: Control, 80; Vinclozolin, 170

F 3: Control, 100; Vinclozolin, 200

F 4: Control, 120; Vinclozolin, 180

The vertical-axis of the second graph is labeled sperm number in millions per milliliter ranging from 0 to 40 with intervals of 5. Approximate data from the second graph are as follows:

F 1: Control, 28; Vinclozolin, 21

F 2: Control, 24; Vinclozolin, 17

F 3: Control, 25; Vinclozolin, 23

F 4: Control, 34; Vinclozolin, 26

The vertical-axis of the third graph is labeled sperm forward motility average in percentage ranging from 0 to 90 percent with intervals of 10. Approximate data from the third graph are as follows:

F 1: Control, 73; Vinclozolin, 60

F 2: Control, 68; Vinclozolin, 40

F 3: Control, 72; Vinclozolin, 56

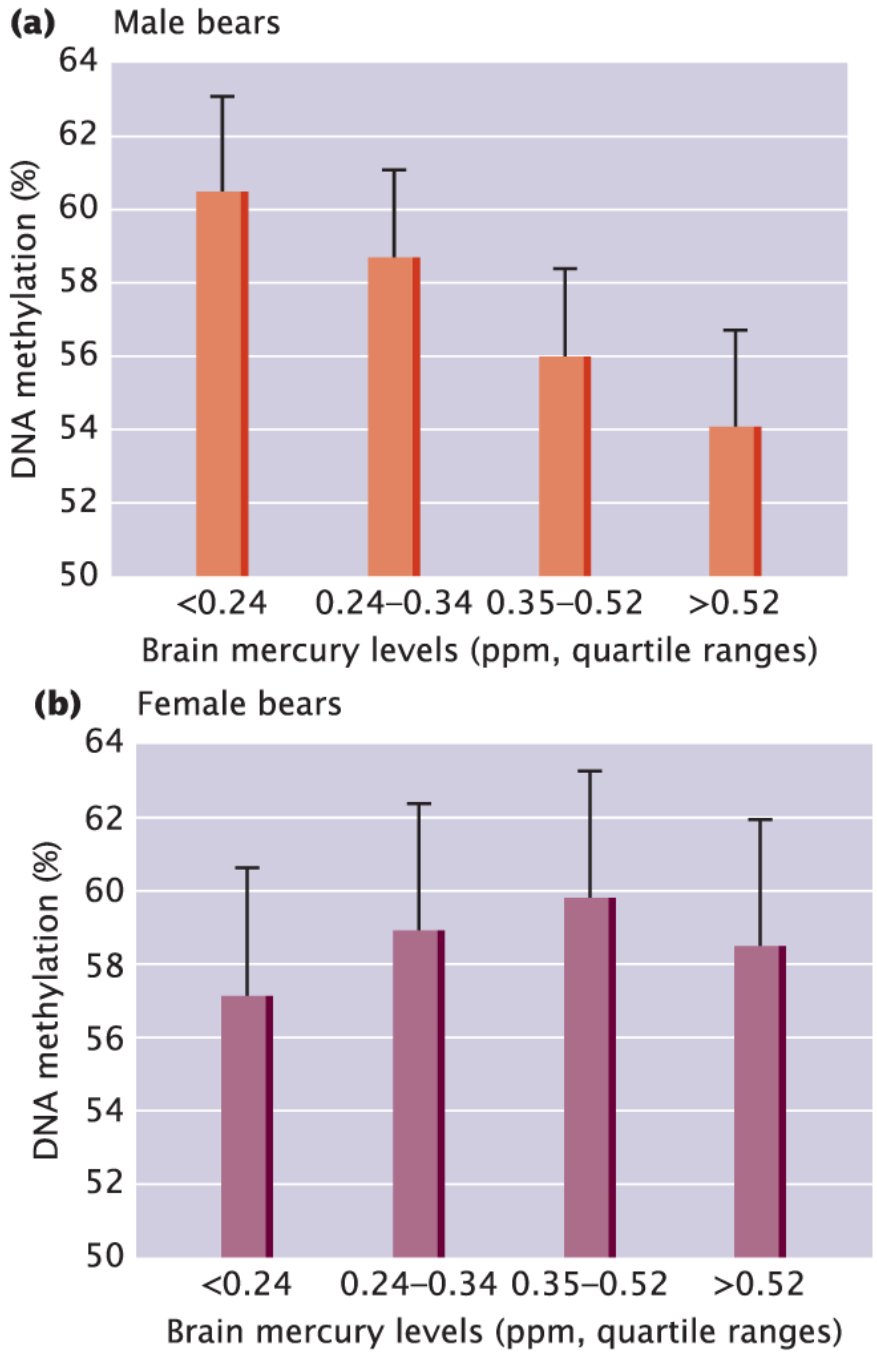
F 4: Control, 79; Vinclozolin, 58

All of the vinclozolin bars have an asterisk above them, which indicates their p value is less than 0 point 0 0 1.

33. Based on the information from studies of the long-term effects of diet on metabolism in mice, what might the epigenetic effects be on the children and grandchildren of people who were exposed to famine as children? Include in your answer the types of epigenetic changes in chromatin that you might expect to see and the phenotypic effects on lipid and cholesterol metabolism.

34.

Mercury is a major pollutant in arctic ecosystems, and polar bears are particularly vulnerable to mercury contamination because it concentrates in the food they eat and then in their tissues. Some chemical pollutants are known to alter gene expression, but the mechanisms of these effects are unknown. Pilsner et al. 2010 (*Molecular Ecology* 19:307–314) were interested in whether mercury might affect gene expression in polar bears through epigenetic modifications. They examined levels of mercury and genome-wide levels of DNA methylation in brain tissues from 47 polar bears collected by local Inuit subsistence hunters. They measured DNA methylation with a technique called LUminometric Methylation Assay (LUMA), which uses methyl-sensitive and methyl-insensitive restriction endonucleases, followed by PCR and DNA sequencing, to measure genome-wide DNA methylation. The results for male and female polar bears are shown on the right. What conclusions can you draw from the data about epigenetic changes associated with mercury pollution in polar bears? What predictions can you make about how gene expression in polar bears might be affected by mercury pollution?



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[Data from Pilsner et al. 2010. *Molecular Ecology* 19:307–314.]

Description

The horizontal axis of both the graphs show brain mercury levels in p p m, quartile ranges and has the following labeling: less than 0 point 2 4, 0 point 2 4 to 0 point 3 4, 0 point 3 5 to 0 point 5 2, and greater than 0 point 5 2. The vertical axis of both the graphs represents D N A methylation in percent ranging from 50 to 64 with intervals of 2. The first graph is labeled male bears and shows the following approximate data:

Less than 0 point 2 4: 60 point 2, the bar is extending to 63.

0 point 2 4 to 0 point 3 4: 58 point 5, the bar is extending to 61.

0 point 3 5 to 0 point 5 2: 56, the bar is extending to 58 point 5.

Greater than 0 point 5 2: 54, the bar is extending to 57.

The second graph is labeled female bears and shows the following approximate data:

Less than 0 point 2 4: 57, the bar is extending to 60 point 5.

0 point 2 4 to 0 point 3 4: 59, the bar is extending to 62 point 5.

0 point 3 5 to 0 point 5 2: 60, the bar is extending to 63 point 5.

Greater than 0 point 5 2: 58 point 5, the bar is extending to 62.

CHALLENGE QUESTIONS

Section 21.3

35.

In recent years, techniques have been developed to clone mammals through a process called nuclear transfer, in which the nucleus of a somatic cell is transferred to an

egg cell from which the nuclear material has been removed (see Chapter 22). Research has demonstrated that when a nucleus from a differentiated somatic cell is transferred to an egg cell, only a small percentage of the resulting embryos complete development, and many of those that do die shortly after birth. In contrast, when a nucleus from an undifferentiated embryonic stem cell is transferred to an egg cell, the percentage of embryos that complete development is significantly higher (W. M. Rideout, K. Eggan, and R. Jaenisch. 2001. *Science* 293:1095–1098). Propose a possible reason for why a higher percentage of cloned embryos develop successfully when the nucleus transferred comes from an undifferentiated embryonic stem cell.

36. Would the genetic conflict hypothesis be likely to explain genomic imprinting for genes involved in adult memory? Why or why not?

37. The use of embryonic stem cells has been proposed for replacing cells that are destroyed by disease or injury. Because of ethical concerns about creating and destroying embryos to produce embryonic stem cells, researchers have attempted to create induced pluripotent stem cells (iPSCs). In this chapter, we discussed studies showing that iPSCs retain some epigenetic marks from the differentiated adult cells from which they were derived. What implications might this research have for attempts to use iPSCs to regrow cells and tissues?

🗣️ ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 21.1

1. Epigenetics has been described as “inheritance, but not as we know it.” Do you think this is a good definition? Why or why not?

Section 21.2

2. One reason that worker bees forgo their own reproduction to help their sister (the queen) reproduce is that female bees are more closely related to their sisters than they are to their own offspring. This quirk of genetics results from the fact that bees have haplodiploid sex determination, in which females are diploid, with a mother and a father, but males are haploid, developing from unfertilized eggs. Because males are haploid, they produce sperm by mitosis. Explain why haplodiploid sex determination causes females to be more closely related to their sisters than to their offspring.

Section 21.3

3. How are epialleles different from genetic alleles, such as those that encode differences in the shape of peas or blood types?

4. How is paramutation similar to normal gene mutation? How does it differ? Make a list of similarities and differences.

5. Genomic imprinting occurs when alleles inherited from male and female parents are expressed differently in the offspring. The genetic conflict hypothesis provides an explanation for genomic imprinting in terms of natural selection (see Chapter 25). Based on the genetic conflict hypothesis, would you expect to see higher expression of paternal or maternal alleles for a locus that encodes a fetal growth enhancer? What about the expression of paternal and maternal alleles for a locus that encodes a fetal growth suppressor? Explain your answer in terms of the genetic conflict hypothesis.

6. People often say that monozygotic twins are genetically identical. Do you think that is a correct statement? Present arguments for and against this statement.

Section 21.4

7. In the introduction to this chapter, we discussed the role of famine during early prenatal development and how, through epigenetic effects, it influenced the health of individuals as adults. One effect that has been observed in people who were conceived during the Dutch Hunger Winter is that they suffer more cognitive decline later in life. If these individuals were autopsied after death, what differences might you expect to see in the chromatin of their brain cells? What would the mouse studies of cognition and epigenetic effects suggest?

8. Some people have said that epigenetics provides genes with a memory. Explain how epigenetics allows genes to remember.

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Draw a concept map that outlines how epigenetic changes potentially contributed to obesity, stroke, and heart disease among people who were conceived during the Dutch Hunger Winter. Include the following terms and phrases in your map: Dutch Hunger Winter, starvation, caloric restriction during intrauterine development, chromatin structure, DNA methylation, epigenetic alterations, loci involved in growth and metabolic disease, thrifty phenotype hypothesis, obesity, stroke, cardiovascular disease.

Section 21.2

2. Create a concept map that summarizes the different types of molecular changes that lead to epigenetic alterations and the effects these alterations have on gene expression.

Section 21.3

3. Draw a concept map that explains paramutation at the *b1* locus in corn. Include at least the following terms and phrases in your map: paramutation, *b1* locus, epigenetic modification, heritable change in gene expression, *B-I* allele, B' allele, B'^{*} allele, epialleles, levels of anthocyanin, tandem repeats, open

chromatin, closed chromatin, high levels of transcription, and low levels of transcription.

4. Create a concept map that outlines the epigenetic effects that bring about X in activation, including the different genes involved and their actions.

Section 21.4

5. Use a concept map to outline the epigenetic effects of licking and grooming behavior on the response to fear and stress in rats. Include at least the following terms and concepts in your map: rats, maternal behavior, response to fear and stress, licking and grooming of offspring, hormonal response to stress, DNA methylation, acetylation of histone proteins, expression of glucocorticoid receptor gene, infusion of brains, deacetylase inhibitor.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 22

Developmental Genetics and Immunogenetics

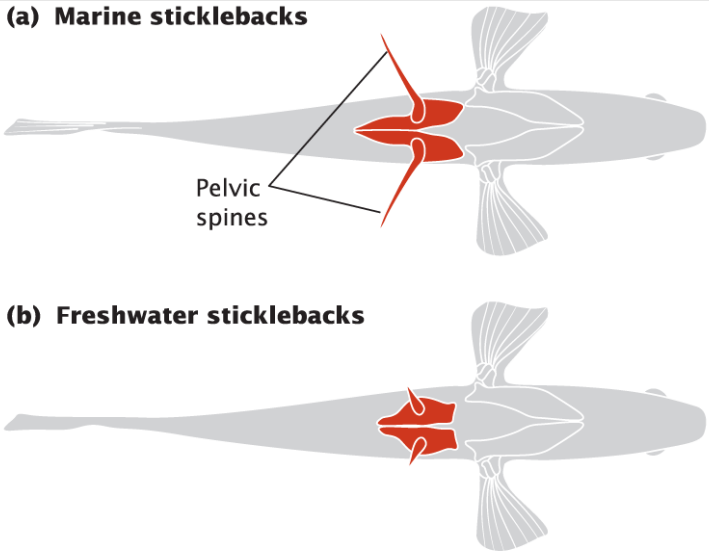
Barrett & MacKay/Getty Images.

Alterations of key regulatory sequences can bring about major developmental changes. Mutations in an enhancer of the *Pitx1* gene have caused the loss of pelvic spines in freshwater populations of threespine sticklebacks (shown here).

The Origin of Spineless Sticklebacks

Threespine sticklebacks (*Gasterosteus aculeatus*) are curious little fish. In spite of their small size—they reach only about 2 inches (5

cm) in length—they are heavily armored, with protective plates on the back, sides, and belly, and possess three spines on the dorsal surface, thus giving rise to their name. Each fish also possesses two impressive pelvic spines, which are anchored to the pelvic girdle and project out from the sides (Figure 22.1). The dorsal and pelvic spines make sticklebacks difficult to swallow, allowing them to survive in an environment where an unprotected 2-inch fish is an easily caught meal for numerous predators.



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22.1 Marine populations of threespine sticklebacks possess pelvic spines (a), but the spines are reduced or missing in sticklebacks from many freshwater lakes (b). [Information from Macmillan Publishers Ltd: Neil H. Shubin and Randall D. Dahn, Evolutionary biology: Lost and found, *Nature* 428:703–704, 2004, copyright 2004.]

Description
The marine stickleback shows long pelvic spines extending outside the body. The freshwater stickleback shows shorter pelvic spines which remain inside the body.

Most sticklebacks are marine, living in the ocean, but a few isolated populations can be found inland, in freshwater lakes. In North America, these freshwater populations originated 10,000 to 20,000 years ago, at the end of the last ice age, when marine sticklebacks invaded the lakes. Many lake populations of threespine sticklebacks have lost their armor and spines, probably because fish predators that might eat them are absent from the lakes, there is little calcium available to develop the plates and spines, and invertebrate predators found in the lakes catch the fish by grabbing onto their spines. Biologists have long been interested in how sticklebacks made the evolutionary transition from the marine to the freshwater environment: How did a heavily armored fish become spineless? Research conducted by developmental geneticists has begun to provide an answer to this question.

In 1998, geneticist David Kingsley, from Stanford University, began a collaboration with Dolph Schluter, an evolutionary biologist from the University of British Columbia. Their goal was to understand how threespine sticklebacks lost their pelvic spines during the evolutionary transition from marine to freshwater environments. The scientists crossed a female marine stickleback that possessed spines with a male from Paxton Lake, British Columbia, that lacked pelvic spines. All of the F_1 fish from this cross possessed pelvic spines. They then crossed two of the F_1 fish, producing a total of 375 F_2 progeny. These F_2 progeny showed a wide range of variation in their pelvic spines: some had fully developed spines, some lacked spines altogether, and others had varying degrees of spine reduction.

Kingsley and his colleagues then examined the association of pelvic spines in the F_2 progeny with their inheritance of genetic markers across the genome. They found that most of the variation in pelvic spines was associated with genetic markers from a particular region on chromosome 7. Interestingly, this same region contains *Pitx1*, a gene that is often expressed in the hind limbs of developing vertebrates. Mice with a mutation in the *Pitx1* gene often have reduced hind limbs as well as other developmental abnormalities. This observation suggested that mutations within the *Pitx1* gene might be responsible for the absence of pelvic spines in freshwater populations of sticklebacks. But when the researchers examined the DNA sequences of the *Pitx1* gene in marine and freshwater threespine sticklebacks, they found no differences that would alter the amino acid sequence of the protein encoded by the gene. What they did find was that the expression of the *Pitx1* gene differed in marine and freshwater fish. Sticklebacks with pelvic spines expressed *Pitx1* in the pelvic region; sticklebacks without pelvic spines expressed *Pitx1* in other tissues, but the gene was completely inactive in the pelvic region. This finding suggested that although the *Pitx1* gene itself is not mutated in spineless fish, mutations in regulatory elements that affect the expression of *Pitx1* might be the source of variation in the presence or absence of pelvic spines.

In 2010, Kingsley and his team located the mutation that causes the absence of spines in freshwater populations of threespine sticklebacks. They found an enhancer 500 bp upstream of the *Pitx1*

gene that controls the tissue-specific expression of *Pitx1*. Enhancers are DNA sequences that promote the transcription of distant genes, often in a tissue-specific manner (see Chapter 17). The researchers determined that spineless fish from Paxton Lake possessed a deletion that removed this enhancer, thus preventing expression of *Pitx1* and ultimately resulting in the loss of pelvic spines. Spineless fish from other lakes in Canada, Alaska, and even Iceland also possessed deletions of the same enhancer, but fish from different lakes possessed different deletions. This observation suggests that during the course of evolution, pelvic spines have been lost multiple times through natural selection acting on different mutations that have the same phenotypic effect.

THINK-PAIR-SHARE

- Some uninformed people might argue that studying the development of an obscure little fish like the threespine stickleback is a waste of time and money. Why would biologists study the genes that control development in stickleback fish? Give some reasons.
- Threespine sticklebacks that invaded freshwater lakes have lost their pelvic spines. What other adaptations might you expect to see in fish that have moved from a marine environment to a freshwater habitat?

The story of how the stickleback lost its spines illustrates that major anatomical alterations can occur through small genetic changes in key regulatory sequences that affect development, a theme throughout this chapter on the genetic control of development. The chapter begins with a consideration of how cell differentiation

occurs, not through loss of genes but rather through alteration of gene expression. We then discuss the genetic control of early development of *Drosophila* embryos, one of the best-understood developmental systems. We next consider the genetic control of floral structure in plants, another model system that has been well studied, and then take a more detailed look at programmed cell death and the use of development for understanding evolution. At the end of the chapter, we turn to the development of immunity and its genetic control.

22.1 Development Takes Place Through Cell Determination

Every multicellular organism begins life as a unicellular fertilized egg. This single-celled zygote undergoes repeated cell divisions, eventually producing millions or trillions of cells that constitute a complete adult organism. Initially, each cell in the early embryo is **totipotent**: it has the potential to develop into any cell type. Many cells in plants and fungi remain totipotent, but animal cells usually become committed to developing into specific types of cells after just a few early embryonic divisions. This commitment often comes well before a cell begins to exhibit any characteristics of a particular cell type; when the cell becomes committed, it does not normally reverse its fate and develop into a different cell type. A cell becomes committed by a process called **determination** (see discussion of stem cells and differentiation in Chapter 21, pp. 658–659).

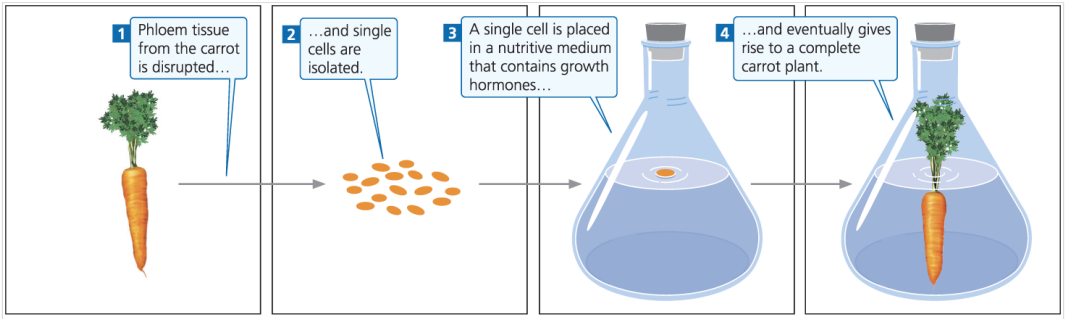
For many years, the work of developmental biologists was limited to describing the changes that take place in the course of development, because techniques for probing the intracellular processes behind these changes were not available. But in recent years, powerful genetic and molecular techniques have had a tremendous influence on the study of development; for example, DNA sequencing has provided a great deal of information about the nature and organization of DNA sequences that control developmental processes. In some model systems, such as *Drosophila* and

Arabidopsis, the molecular mechanisms underlying developmental change are now beginning to be understood.

If all cells in a multicellular organism are derived from the same original cell, how do different cell types arise? Before the 1950s, two hypotheses were considered. One possibility was that throughout development, genes might be selectively lost or altered, causing different cell types to have different genomes. Alternatively, each cell might contain the same genetic information, but different genes might be expressed in each cell type. The results of early cloning experiments helped settle this issue.

Cloning Experiments on Plants

In the 1950s, Frederick Steward developed methods for cloning plants. He disrupted phloem tissue from the root of a carrot by separating and isolating individual cells, and then placed individual cells in a sterile medium that contained nutrients and other substances required for growth. Steward was successful in getting the cells to grow and divide, and he eventually obtained whole edible carrots from single cells (**Figure 22.2**). Because all parts of the plant were regenerated from a specialized phloem cell, he concluded that each phloem cell contained the genetic potential for a whole plant: none of the original genetic material was lost during determination.



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22.2 Many kinds of plants can be cloned from isolated single cells. This type of experiment demonstrates that none of the original genetic material is lost during plant development.

Description

The illustration shows a carrot from which single cells are isolated. A caption reads, “Phloem tissue from the carrot is disrupted and single cells are isolated.” One single cell is placed inside a liquid medium in a flask. The illustration then shows a carrot in the flask. A caption reads, “A single cell is placed in a nutritive medium that contains growth hormones and eventually gives rise to a complete carrot plant.”

Cloning Experiments on Animals

The results of other studies demonstrated that most animal cells also retain a complete set of genetic information during development. In 1952, Robert Briggs and Thomas King removed the nuclei from unfertilized oocytes of the frog *Lithobates (Rana) pipiens.* They then isolated nuclei from frog blastulas (an early embryonic stage), injected these nuclei individually into the oocytes, and pricked the eggs with a needle to stimulate them to divide. Although

most were damaged in the process, a few eggs developed into complete tadpoles that eventually metamorphosed into frogs.

In the late 1960s, John Gurdon used these methods to successfully clone a few frogs with nuclei isolated from the intestinal cells of tadpoles. This result suggested that the differentiated intestinal cells carried the genetic information necessary to encode traits found in all other cells. However, Gurdon's successful clonings may have resulted from the presence of a few undifferentiated stem cells in the intestinal tissue, which were inadvertently used as nucleus donors.

In 1997, researchers at the Roslin Institute of Scotland announced that they had successfully cloned a sheep using the genetic material from a differentiated cell of an adult animal. To perform this experiment, they fused an udder cell from a white-faced Finn Dorset ewe with an enucleated egg cell and stimulated the egg electrically to initiate development. After growing the embryo in the laboratory for a week, they implanted it into a Scottish black-faced surrogate mother. Dolly, the first mammal cloned from an adult cell, was born on July 5, 1996 (**Figure 22.3**). Since then, a number of other animals, including sheep, goats, mice, rabbits, cows, pigs, horses, mules, dogs, and cats, have been cloned from differentiated adult cells. Importantly, although Dolly and other cloned mammals contain the same *nuclear* genetic material as their donor-cell parent, they are not identical for *cytoplasmic* genes, such as those on the mitochondrial

chromosome, because the cytoplasm is donated by both the donor cell and the enucleated egg cell.

AP Photo/Paul Clements.

22.3 In 1996, researchers at the Roslin Institute of Scotland successfully cloned a sheep named Dolly. They used the genetic material from a differentiated cell of an adult animal.

These cloning experiments demonstrated that genetic material is not lost or permanently altered during development; therefore, development must require the selective expression of genes. But how do cells regulate their gene expression in a coordinated manner to give rise to a complex multicellular organism? Research has now begun to provide some answers to this important question.

THINK-PAIR-SHARE Questions 1 and 2

CONCEPTS

The successful cloning of plants and animals from single differentiated cells demonstrates that genes are not lost or permanently altered during development.

✔ CONCEPT CHECK 1

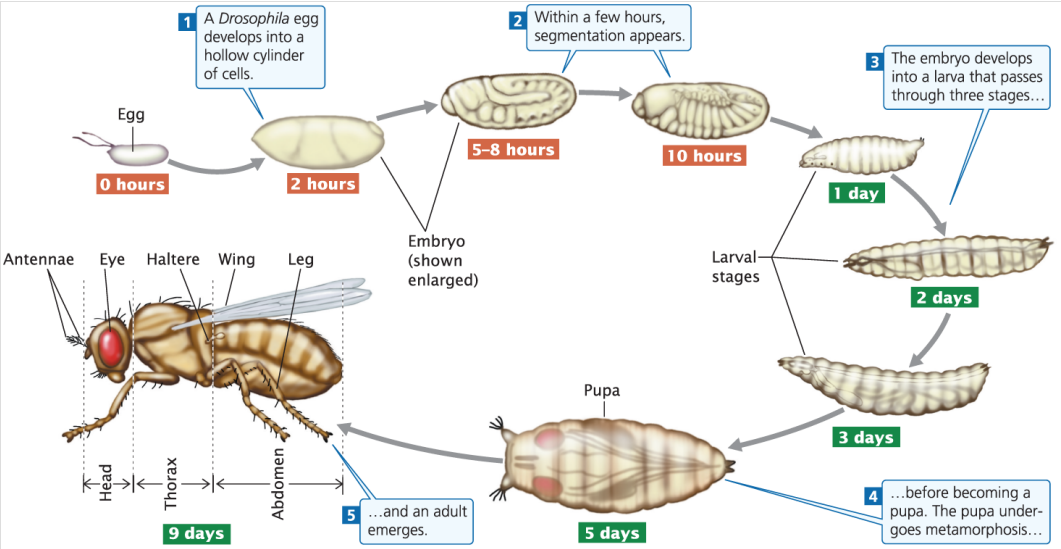
Scientists have cloned some animals by injecting a nucleus from an early embryo into an enucleated egg cell. Does this outcome demonstrate that genetic material is not lost during development? Why or why not?

22.2 Pattern Formation in *Drosophila* Serves as a Model for the Genetic Control of Development

Pattern formation consists of the developmental processes that lead to the shape and structure of complex multicellular organisms. One of the best-studied systems for the genetic control of pattern formation is the early embryonic development of *Drosophila melanogaster*. Geneticists have isolated a large number of mutations in fruit flies that influence all aspects of their development, and molecular analysis of these mutations has provided a great deal of information about how genes control early development in *Drosophila*.

The Development of the Fruit Fly

An adult fruit fly possesses three basic body parts: head, thorax, and abdomen (**Figure 22.4**). The thorax consists of three segments: the first thoracic segment carries a pair of legs; the second thoracic segment carries a pair of legs and a pair of wings; and the third thoracic segment carries a pair of legs and the halteres (rudiments of the second pair of wings found in most other insects). The abdomen consists of eight segments.



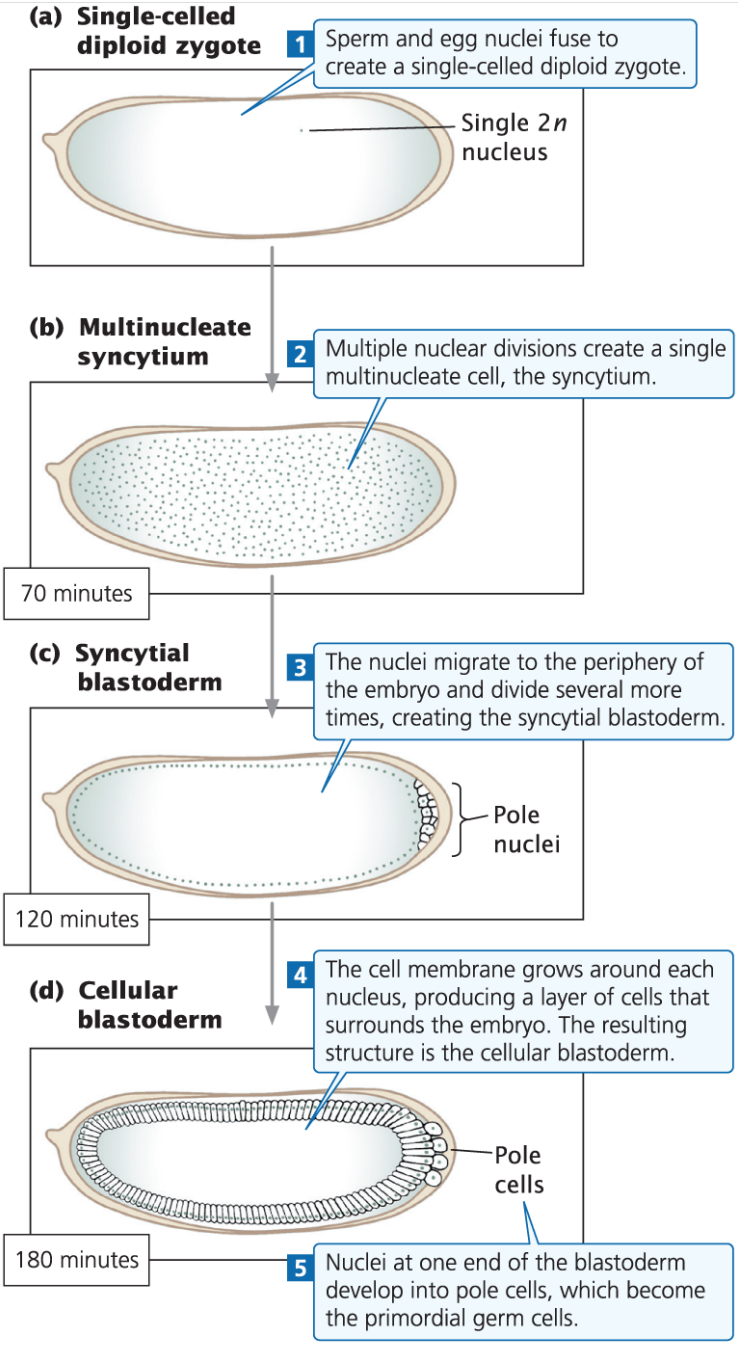
22.4 The fruit fly *Drosophila melanogaster* passes through three larval stages and a pupal stage before developing into an adult fly. The three major body parts of the adult are head, thorax, and abdomen.

Description

The life cycle starts with an egg at 0 hours. It shows a large egg at 2 hours. A caption reads, “A Drosophila egg develops into a hollow cylinder of cells.” The large egg shows segmentation at 5 to 8 hours. Even more segmentation occurs at 10 hour. A caption reads, “Within a few hours, segmentation appears.” After 1 day, a larva is formed. A caption reads, “The embryo develops into a larva that passes through three stages before becoming a pupa. The pupa undergoes metamorphosis and an adult emerges.” The larva grows and enlarges at days 2 and 3. At day 5, a pupa is formed showing differentiation in body. At day 9, an adult Drosophila melanogaster is shown. Its body is differentiated into head, thorax, and abdomen. It has antennae and eye in the head region. The thorax region shows a haltere. The abdomen shows wings and legs.

When a *Drosophila* egg has been fertilized, its diploid nucleus (Figure 22.5a) immediately divides nine times without division of the cytoplasm, creating a single multinucleate cell called the syncytium (Figure 22.5b). These nuclei are scattered throughout the

cytoplasm but later migrate toward the periphery of the embryo and divide several more times (**Figure 22.5c**). Next, the cell membrane grows inward and around each nucleus, creating a layer of approximately 6000 cells at the outer surface of the embryo (**Figure 22.5d**). Nuclei at one end of the embryo develop into pole cells, which eventually give rise to germ cells.



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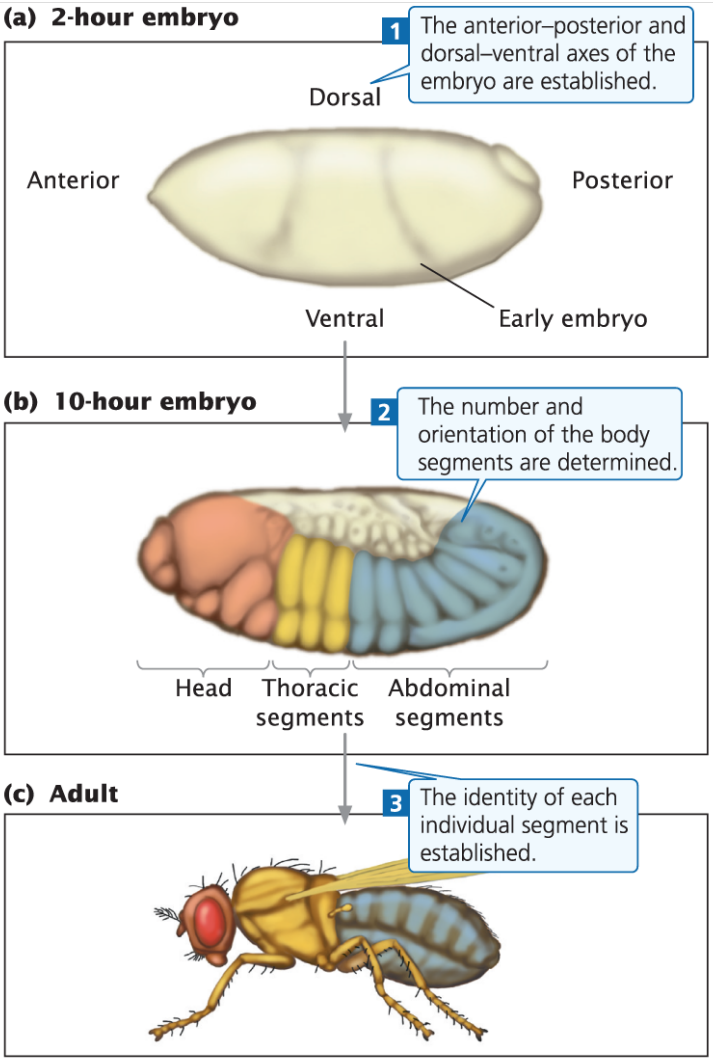
22.5 Early development of a *Drosophila* embryo.

Description

Part “a” labeled single-celled diploid zygote shows an oval shaped cell with a large nucleus labeled single 2 n nucleus. A caption reads, “Sperm and egg nuclei fuse to create a single-

celled diploid zygote." Part "b" labeled multinucleate syncytium shows several dots in the nucleus at 70 minutes. A caption reads, "Multiple nuclear divisions create a single multinucleate cell, the syncytium." Part "c" labeled syncytial blastoderm shows dots arranged peripherally and pole nuclei at one end at 120 minutes. A caption reads, "The nuclei migrate to the periphery of the embryo and divide several more times, creating the syncytial blastoderm." Part "d" labeled cellular blastoderm shows dots grown into elongated structures at the periphery and four pole cells are shown at the end at 180 minutes. A caption reads, "The cell membrane grows around each nucleus, producing a layer of cells that surrounds the embryo. The resulting structure is the cellular blastoderm." A caption pointing towards pole cells reads, "Nuclei at one end of the blastoderm develop into pole cells, which become the primordial germ cells."

The early embryo then undergoes further development in three distinct stages: (1) the anterior–posterior axis and the dorsal–ventral axis of the embryo are established (Figure 22.6a), (2) the number and orientation of the body segments are determined (Figure 22.6b), and (3) the identity of each individual segment is established (Figure 22.6c). Different sets of genes control each of these three stages (Table 22.1).



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22.6 In an early *Drosophila* embryo, the major body axes are established, the number and orientation of the body segments are determined, and the identity of each individual segment is established. Different sets of genes control each of these three stages.

Description

Part “a” shows a 2-hour embryo, an oval shaped structure labeled early embryo. The top is labeled dorsal, bottom is labeled ventral, right side is labeled posterior, and left side is labeled anterior. A caption reads, “The anterior–posterior and dorsal–ventral axes of the embryo are established.” Part “b” shows a 10-hour embryo with segmentation in the early embryo. The head, thoracic and abdominal segments are present. A caption reads, “The

number and orientation of the body segments are established." Another caption reads, "The identity of each individual segment is established." Part "c" shows a diagram of an adult Drosophila.

TABLE 22.1 Stages in the early development of fruit flies and the genes that control each stage

Developmental Stage	Genes
Establishment of main body axes	Egg-polarity genes
Determination of number and orientation of body segments	Segmentation genes
Establishment of identity of each segment	Homeotic genes

Egg-Polarity Genes

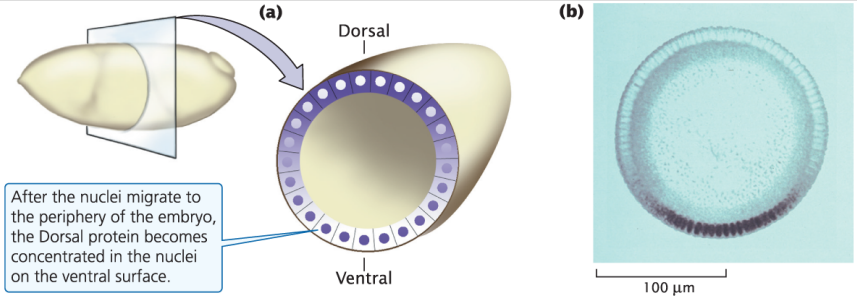
The **egg-polarity genes** (genes that determine polarity, or direction) play a crucial role in establishing the two main axes of development in fruit flies. You can think of these axes as the longitude and latitude of development: any location in the *Drosophila* embryo can be defined in relation to these two axes.

The egg-polarity genes are transcribed into mRNAs in the course of egg formation (oogenesis) in the maternal parent, and these mRNAs become incorporated into the cytoplasm of the egg. The mRNAs are translated into proteins that play an important role in determining the anterior–posterior and dorsal–ventral axes of the embryo. Because the mRNAs of the polarity genes are produced by the female parent and influence the phenotype of the offspring, the traits encoded by them are examples of genetic maternal effect (see Chapter 5).

There are two sets of egg-polarity genes: one set determines the anterior–posterior axis, and the other determines the dorsal–ventral axis. These genes work by setting up concentration gradients of morphogens within the developing embryo. A **morphogen** is a protein that varies in concentration and elicits different developmental responses at different concentrations. Egg-polarity genes function by producing proteins that become asymmetrically distributed in the cytoplasm, giving the egg polarity. This asymmetrical distribution may take place in a couple of ways. An mRNA may be localized to particular regions of the egg cell, leading to an abundance of the protein in those regions when the mRNA is translated. Alternatively, the mRNA may be randomly distributed, but the protein that it encodes may become asymmetrically distributed by a transport system that delivers it to particular regions of the cell, by regulation of its translation, or by its removal from particular regions by selective degradation.

DETERMINATION OF THE DORSAL–VENTRAL AXIS

The dorsal–ventral axis defines the back (dorsum) and belly (ventrum) of a fly (see Figure 22.6). At least 12 different genes determine this axis, one of the most important being a gene called *dorsal*. The *dorsal* gene is transcribed and translated in the maternal ovary, and the resulting mRNA and protein are transferred to the egg during oogenesis. In a newly laid egg, mRNA and protein encoded by the *dorsal* gene are uniformly distributed throughout the cytoplasm, but after the nuclei have migrated to the periphery of the embryo (see Figure 22.5c), Dorsal protein becomes redistributed. Along one side of the embryo, Dorsal protein remains in the cytoplasm; this side will become the dorsal surface. Along the other side, Dorsal protein is taken up into the nuclei; this side will become the ventral surface. At this point, there is a smooth gradient of increasing nuclear Dorsal concentration from the dorsal to the ventral side (**Figure 22.7**).



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Part b: Max Planck Institute for Developmental Biology.

22.7 Dorsal protein in the nuclei helps to determine the dorsal–ventral axis of the *Drosophila* embryo. (a) Relative concentrations of Dorsal protein in the cytoplasm and nuclei of cells in the early *Drosophila* embryo. (b) Micrograph of a

cross section of an embryo showing the Dorsal protein, darkly stained, in the nuclei along the ventral surface.

Description
Part “a” shows the cross-section of a Drosophila embryo. It shows a dot-like structure in the periphery. Dorsal and ventral are labeled at the top and bottom of the cross-section. A caption reads, “After the nuclei migrate to the periphery of the embryo, the Dorsal protein becomes concentrated in the nuclei on the ventral surface.” Part “b” shows a micrograph of the cross-section depicting dorsal protein. A scale at the bottom reads 100 microgram.

The nuclear uptake of Dorsal protein is thought to be governed by a protein called Cactus, which binds to Dorsal protein and traps it in the cytoplasm. The presence of yet another protein, called Toll, leads to the phosphorylation of Cactus, causing it to be degraded. Where Cactus is degraded, Dorsal is released and can move into the nucleus. Together, Cactus and Toll regulate the nuclear distribution of Dorsal protein, which in turn determines the dorsal–ventral axis of the embryo.

Inside the nucleus, Dorsal protein acts as a transcription factor, binding to regulatory sites on the DNA and activating or repressing the expression of other genes (Table 22.2). High nuclear concentrations of Dorsal protein (as in cells on the ventral side of the embryo) activate a gene called *twist*, which causes ventral tissues to develop. Low nuclear concentrations of Dorsal protein (as in cells on the dorsal side of the embryo) activate a gene called *decapentaplegic*, which specifies dorsal structures. In this way, the ventral and dorsal sides of the embryo are determined.

TABLE 22.2 Key genes that control the development of the dorsal–ventral axis in fruit flies and their action

Gene	Where Expressed	Action of Gene Product
dorsal	Ovary	Affects the expression of genes such as *twist* and *decapentaplegic*
cactus	Ovary	Traps Dorsal protein in the cytoplasm
toll	Ovary	Leads to the phosphorylation of Cactus, which is then degraded, releasing Dorsal to move into the nuclei of ventral cells
twist	Embryo	Takes part in the development of mesodermal tissues*
decapentaplegic	Embryo	Takes part in the development of gut structures

*One of the three primary tissue layers in the early embryo

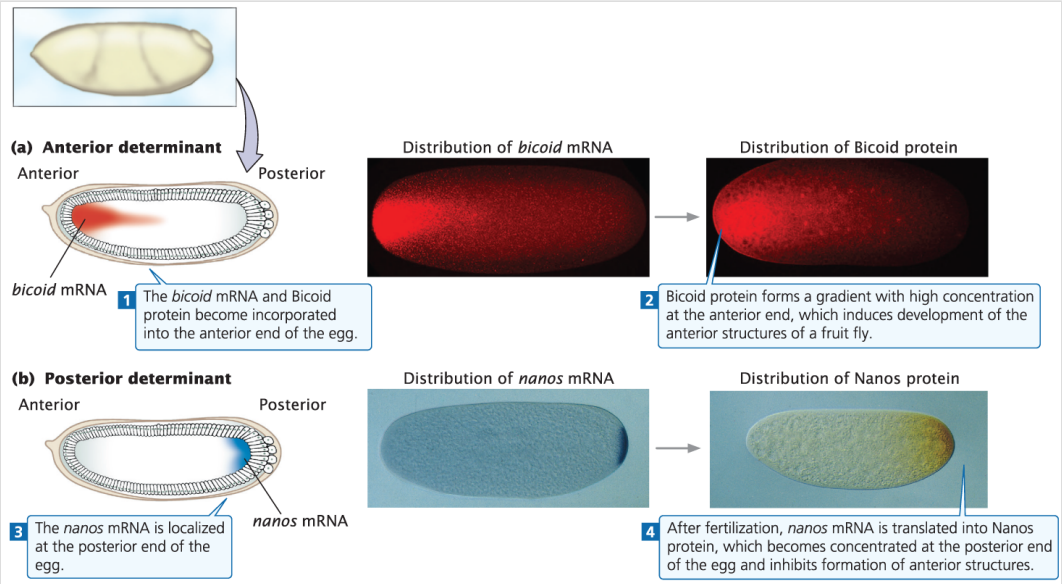
DETERMINATION OF THE ANTERIOR–POSTERIOR AXIS

One of the most important early developmental events is the determination of the anterior (head) and posterior (butt) ends of an animal. Here, we consider several key genes that establish the anterior–posterior axis in the *Drosophila* embryo (Table 22.3). An important gene in this regard is *bicoid*, which is first transcribed in the maternal ovary during oogenesis. The *bicoid* mRNA and Bicoid protein become incorporated into the anterior end of the cytoplasm of the egg. After fertilization, *bicoid* mRNA and Bicoid protein form a concentration gradient along the anterior–posterior axis of the embryo, with high concentrations at the anterior end and lower concentrations at the posterior end (Figure 22.8a). The high concentration of Bicoid protein at the anterior end induces the development of anterior structures, such as the head of the fruit fly. It stimulates the development of anterior structures by binding to regulatory sequences in the DNA and influencing the expression of other genes. One of the most important genes stimulated by Bicoid protein is *hunchback*, which is required for the development of the head and thoracic structures of the fruit fly.

TABLE 22.3 Some key genes that determine the anterior–posterior axis in fruit flies

Gene	Where Expressed	Action

bicoid	Ovary	Regulates expression of genes responsible for anterior structures; stimulates *hunchback*
nanos	Ovary	Regulates expression of genes responsible for posterior structures; inhibits translation of *hunchback* mRNA
hunchback	Embryo	Regulates transcription of genes responsible for anterior structures



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Part a (center, right): Ali-Murthy, Z. and T. B. Kornberg (2016) Bicoid gradient formation and function in the *Drosophila* pre-syncytial blastoderm. *eLIFE:* DOI: dx.doi.org/10.7554/eLife.13222; Published and distributed under the terms of the Creative Commons Attribution License; https://creativecommons.org/licenses/by/4.0/. Part b (center, right): Courtesy of E. R. Gavis, L. K. Dickenson, and R. Lehman, Massachusetts Institute of Technology.

22.8 The anterior–posterior axis in a *Drosophila* embryo is determined by concentrations of Bicoid and Nanos proteins.

Description
Part “a” labeled anterior determinant shows an early Drosophila embryo. Its cross-section shows bicoid m R N A as red color extending from the anterior end. Anterior and posterior regions are labeled at left and right end respectively. A caption reads, “The bicoid m R N A and Bicoid protein become incorporated into the anterior end of the egg.” Two micrographs show red colored rays extending from left end to the right. One micrograph is labeled distribution of bicoid m R N A, while the other is labeled distribution of bicoid protein. A caption reads, “Bicoid protein forms a gradient with high concentration at the anterior end, which induces development of the anterior structures of a fruit fl¬y.” Part “b” labeled posterior determinant shows the early embryo with nanos m R N A at the posterior end as blue color. A caption reads, “The nanos m R N A is localized at the posterior end of the egg.” Two micrographs show a blue colored region and an orange colored region at their right ends. One micrograph is labeled distribution of nanos m R N A, while the other is labeled distribution of nanos protein. A caption reads, “After fertilization, nanos m R N A is translated into Nanos protein, which becomes concentrated at the posterior end of the egg and inhibits formation of anterior structures.”

The development of the anterior–posterior axis is also greatly influenced by a gene called *nanos*, an egg-polarity gene that acts at the posterior end of the embryo. The *nanos* gene is transcribed in the ovary of the adult female, and the resulting mRNA becomes localized at the posterior end of the egg (Figure 22.8b). After fertilization, *nanos* mRNA is translated into Nanos protein, which diffuses slowly toward the anterior end. The Nanos protein concentration gradient is opposite that of the Bicoid protein: Nanos is most concentrated at the posterior end of the embryo and is least concentrated at the anterior end. Nanos protein inhibits the formation of anterior

structures by repressing the translation of *hunchback* mRNA. The synthesis of Hunchback protein is therefore stimulated at the anterior end of the embryo by Bicoid protein and repressed at the posterior end by Nanos protein. This combined stimulation and repression results in a Hunchback protein concentration gradient along the anterior–posterior axis that, in turn, affects the expression of other genes and helps determine the anterior and posterior structures.

CONCEPTS

The major axes of development in early fruit-fly embryos are established as a result of initial differences in the distribution of specific mRNAs and proteins encoded by genes in the female parent (genetic maternal effect). These differences in distribution establish concentration gradients of morphogens, which cause different genes to be activated in different parts of the embryo.

✔ CONCEPT CHECK 2

A high concentration of which protein stimulates the development of anterior structures?

a. Dorsal
b. Toll
c. Bicoid
d. Nanos

Segmentation Genes

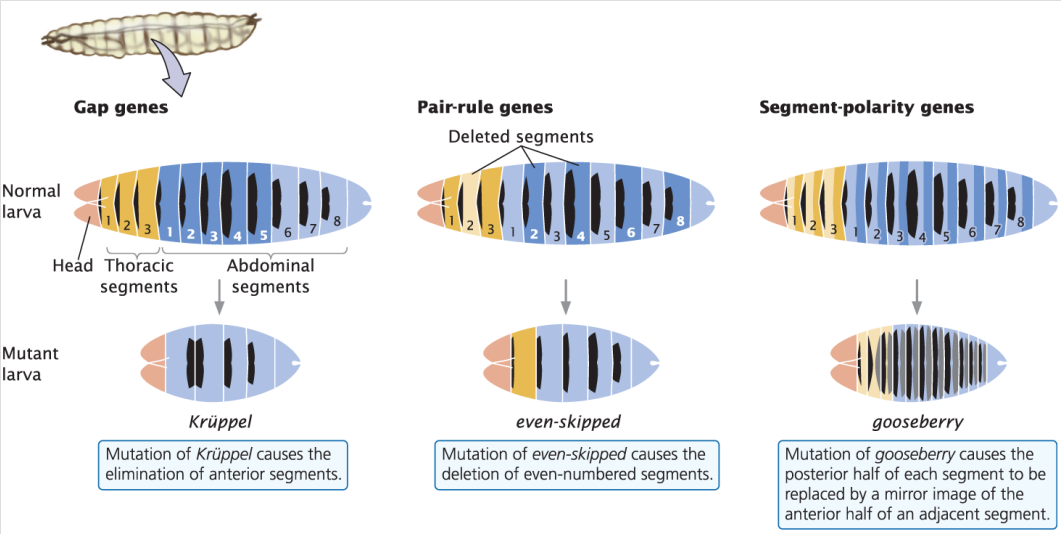
Like all insects, the fruit fly has a segmented body plan. Once the basic dorsal–ventral and anterior–posterior axes of the fruit-fly embryo have been established, **segmentation genes** control the differentiation of the embryo into individual segments. These genes affect the number and organization of the segments, and mutations in them usually disrupt whole sets of segments. The approximately 25 segmentation genes in *Drosophila* are transcribed after fertilization, so they don't exhibit genetic maternal effect, and their expression is regulated by the Bicoid and Nanos protein gradients.

The segmentation genes fall into three classes, as shown in **Table 22.4** and **Figure 22.9**. The three classes act sequentially, affecting progressively smaller regions of the embryo. First, the products of the egg-polarity genes activate or repress **gap genes**, which divide the embryo into broad regions. The gap genes, in turn, regulate **pair-rule genes**, which affect the development of pairs of segments. Finally, the pair-rule genes influence **segment-polarity genes**, which guide the development of individual segments.

TABLE 22.4 Segmentation genes and the effects of mutations in them

Class of Gene	Effect of Mutations	Examples of Genes
Gap genes	Delete groups of adjacent segments	*hunchback, Krüppel, knirps, giant, tailless*

Pair-rule genes	Delete same part of pattern in every other segment	*runt, hairy, fushi tarazu, even-skipped, odd-paired, sloppy paired, odd-skipped*
Segment-polarity genes	Affect polarity of segment; part of segment replaced by mirror image of part of another segment	*engrailed, wingless, gooseberry, cubitus interruptus, patched, hedgehog, disheveled, costal-2, fused*



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22.9 Segmentation genes control the differentiation of the *Drosophila* embryo into individual segments. Gap genes affect large sections of the embryo. Pair-rule genes affect alternate segments. Segment-polarity genes affect the orientation of segments.

Description

The illustration shows three types of larvae which result in mutant larvae. Gap genes are shown in each larva labeled in numbers. The thoracic region shows 3 genes labeled “1” through “3.” The abdominal region shows 8 genes labeled “1” through “8.” The first larva labeled normal larva results in kruppel mutant larva. A caption reads, “Mutation of Kruppel causes the elimination of anterior segments.” The second larva labeled pair-rule genes shows deleted segments in gene 2 in the thoracic region and 2 and 4 in the abdominal region. It leads to an even-skipped mutant larva. A caption reads, “Mutation of even-skipped causes the deletion of even-numbered segments.” The third larva labeled segment-polarity genes shows the same larva as the first one. It leads to gooseberry mutant larva. A caption reads, “Mutation of gooseberry causes the posterior half of each segment to be replaced by a mirror image of the anterior half of an adjacent segment.”

Gap genes define large sections of the embryo; mutations in these genes eliminate whole groups of adjacent segments. Mutations in the *Krüppel* gene, for example, cause the absence of several adjacent segments. Pair-rule genes define regional sections of the embryo and affect alternate segments. Mutations in the *even-skipped* gene cause the deletion of even-numbered segments, whereas mutations in the *fushi tarazu* gene cause the absence of odd-numbered segments. Segment-polarity genes affect the orientation of segments. Mutations in these genes cause part of each segment to be deleted and replaced by a mirror image of part or all of an adjacent segment. For example, mutations in the *gooseberry* gene cause the posterior half of each segment to be replaced by a mirror image of the anterior half of an adjacent segment.

CONCEPTS

Once the major axes of the fruit-fly embryo have been established, segmentation genes determine the number, orientation, and basic organization of the body segments.

✔ CONCEPT CHECK 3

Which of the following is the sequence in which the segmentation genes act?

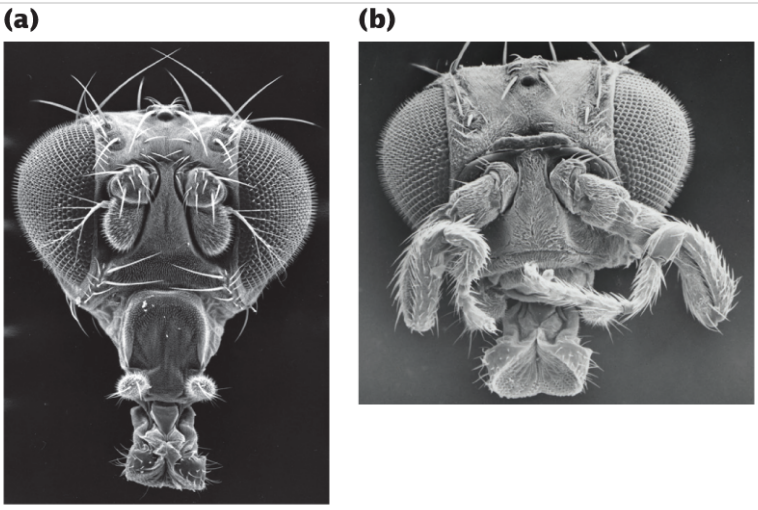
a. Segment-polarity genes → gap genes → pair-rule genes
b. Gap genes → pair-rule genes → segment-polarity genes
c. Segment-polarity genes → pair-rule genes → gap genes
d. Gap genes → segment-polarity genes → pair-rule genes

Homeotic Genes in *Drosophila*

After the segmentation genes have established the number and orientation of the segments, **homeotic genes** become active and determine the *identity* of individual segments. Eyes normally arise only on the head segment, whereas legs develop only on the thoracic segments. The products of homeotic genes activate other genes that encode these segment-specific characteristics. Mutations in the homeotic genes cause body parts to appear in the wrong segments.

In the late 1940s, Edward Lewis began to study homeotic mutations in *Drosophila*—mutations that cause bizarre rearrangements of body parts. Mutations in the *Antennapedia* gene, for example, cause legs to develop on the head of a fly in place of the antennae (**Figure 22.10**). Homeotic genes create addresses for the cells of particular

segments, telling the cells where they are within the regions defined by the segmentation genes. When a homeotic gene is mutated, the address is wrong, and cells in the segment develop as though they were somewhere else in the embryo.



F. Rudolf Turner, Ph.D., Indiana University.

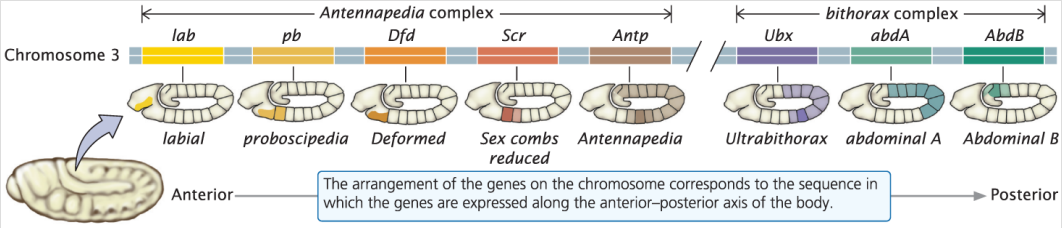
22.10 The homeotic mutation *Antennapedia* substitutes legs for the antennae of a fruit fly. (a) Normal, wild-type antennae. (b) *Antennapedia* mutant.

Homeotic genes in *Drosophila* are expressed after fertilization and are activated by specific concentrations of the proteins produced by the gap, pair-rule, and segment-polarity genes. The homeotic gene *Ultrabithorax* (*Ubx*), for example, is activated when the concentration of Hunchback protein (a product of a gap gene) is within certain values. These concentrations exist only in the middle region of the embryo, so *Ubx* is expressed only in the middle segments.

Most homeotic genes in animals encode transcription factors (see Chapter 17) that bind to DNA and regulate transcription; each of

these genes contains a subset of nucleotides, called a **homeobox**, that is similar in all homeotic genes. The homeobox encodes 60 amino acids that serve as a DNA-binding domain; this domain is related to the helix-turn-helix motif (see Figure 16.2a). Homeoboxes are also present in segmentation genes and other genes that play a role in spatial development.

There are two major clusters of homeotic genes in *Drosophila*. One cluster, the ***Antennapedia* complex**, affects the development of the adult fly's head and anterior thoracic segments. The other cluster, the ***bithorax* complex**, includes genes that influence the adult fly's posterior thoracic and abdominal segments. Together, the *bithorax* and *Antennapedia* gene complexes are termed the **homeotic complex (HOM-C)**. In *Drosophila*, the *bithorax* complex comprises three genes and the *Antennapedia* complex has five; all are located on the same chromosome (**Figure 22.11**). In addition to these eight genes, HOM-C contains many sequences that regulate them. Remarkably, the order of the genes in HOM-C is the same as the order in which the genes are expressed along the anterior–posterior axis of the body. The genes that are expressed in the more anterior segments are found at one end of the complex, whereas those expressed in the more posterior end of the embryo are found at the other end of the complex (see Figure 22.11).



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22.11 Homeotic genes, which determine the identity of individual segments in *Drosophila*, are present in two complexes. The *Antennapedia* complex has five genes, and the *bithorax* complex has three genes.

Description

The illustration shows two complexes on chromosome 3; antennapedia complex and bithorax complex. The genes from left to right in the antennapedia complex are labial (l a b), proboscipedia (p b), deformed (d f d), sex combs reduced (s c r), and antennapedia (a n t p). The genes from left to right in the bithorax complex is ultrabithorax (u b x), abdominal A (a b d A), and abdominal B (a b d B). The arrangement of the genes corresponds to their expression along the anterior-posterior axis. A caption reads, “The arrangement of the genes on the chromosome corresponds to the sequence in which the genes are expressed along the anterior–posterior axis of the body.”

CONCEPTS

Homeotic genes help determine the identity of individual segments in *Drosophila* embryos by producing regulatory proteins that bind to DNA and activate other genes. Each homeotic gene contains a consensus sequence called a homeobox, which encodes the DNA-binding domain.

✔ CONCEPT CHECK 4

Mutations in homeotic genes often cause

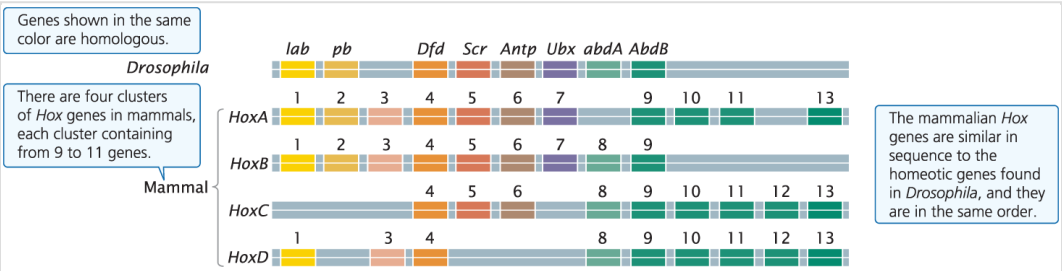
a. the deletion of segments.
b. the absence of structures.
c. too many segments.
d. structures to appear in the wrong place.

Homeobox Genes in Other Organisms

After homeotic genes in *Drosophila* had been isolated and cloned, molecular geneticists set out to determine whether similar genes exist in other animals. Probes complementary to the homeobox of *Drosophila* genes were used to search for homologous genes that might play a role in the development of other animals. The search was hugely successful: homeobox-containing genes have been found in all animals, including nematodes, beetles, sea urchins, frogs, birds, and mammals. Genes with homeoboxes have even been discovered in fungi and plants, indicating that the homeobox arose early in the evolution of eukaryotes. One group of homeobox genes comprises the ***Hox* genes**, which include the homeotic complex of *Drosophila* that we have just described. *Hox* genes are found in all animals except sponges.

In vertebrates, there are usually four clusters of *Hox* genes, each of which contains from 9 to 11 genes. Mammalian *Hox* genes, like those in *Drosophila*, encode transcription factors that help determine the identity of body regions along an anterior–posterior axis. The *Hox* genes of other organisms often exhibit the same relation between order on the chromosome and order of their expression along the anterior–posterior axis of the embryo as that of *Drosophila* (**Figure**

22.12), but this pattern is not universal. For example, the tunicate *Oikopleura dioica* (a primitive relative of vertebrates) has 9 *Hox* genes, but the genes are scattered throughout the genome, in contrast to the clustered arrangement seen in most animals. Despite a lack of physical clustering, the *Hox* genes in *O. dioica* are expressed in the same anterior–posterior order as that seen in vertebrates.



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22.12 *Hox* genes in mammals are similar to those found in *Drosophila*. Here, the complexes are arranged so that genes with similar sequences lie in the same column. See Figure 22.11 for the full names of the *Drosophila* genes.

Description

The drosophila chromosome is on the top D N A segment and 4 mammalian clusters are at the bottom. The drosophila chromosome shows the following genes: lab (corresponding to label 1), p b (corresponding to label 2), D f d (corresponding to label 4), S c r (corresponding to label 5), A n t p (corresponding to label 6), U b x (corresponding to label 7), a b d A (corresponding to label 8), and A b d B (corresponding to label 9). A caption reads, “Genes shown in the same color are homologous.” The mammalian gene clusters are named Hox “A” through Hox “D” and are aligned below drosophila to show their similarity. A caption reads, “There are four clusters of Hox genes in mammals, each cluster containing from 9 to 11 genes.” Hox A shows genes 1 through 7, 9 through 11, and 13. Hox B shows genes 1 through 9. Hox C shows genes 4 through 6 and then 8 through 13. Hox D shows genes 1, 3 and 4, and 8 through 13. A caption reads, “The mammalian Hox genes are similar in sequence to the homeotic genes found in Drosophila, and they are in the same order.”

The *Hox* genes of vertebrates also exhibit a relation between their order on the chromosome and the timing of their expression: genes at one end of the complex (those expressed at the anterior end) are expressed early in development, whereas genes at the other end (those expressed at the posterior end) are expressed later. If a *Hox* gene is experimentally moved to a new location within the *Hox*-gene complex, it is expressed in the appropriate tissue, but the timing of its expression is altered, suggesting that the timing of gene expression is controlled by the physical location of a gene within the complex. Although the mechanism of this sequential control is not well understood, evidence suggests that in mice, it involves a progressive change in the methylation patterns of histone proteins, an epigenetic change that alters chromatin structure and affects transcription (see Chapter 21). Recent studies have also identified microRNA (miRNA) genes (see Section 14.5) within the *Hox*-gene clusters, and evidence suggests that miRNAs play a role in controlling the expression of some *Hox* genes.

THINK-PAIR-SHARE Question 3

Hox genes and their expression are often correlated with anatomical differences among animals, and *Hox* genes are believed to play an important role in the evolution of animals. For example, the lancet *Branchiostoma* (another primitive relative of vertebrates) has a simple body form and possesses only 14 *Hox* genes in a single

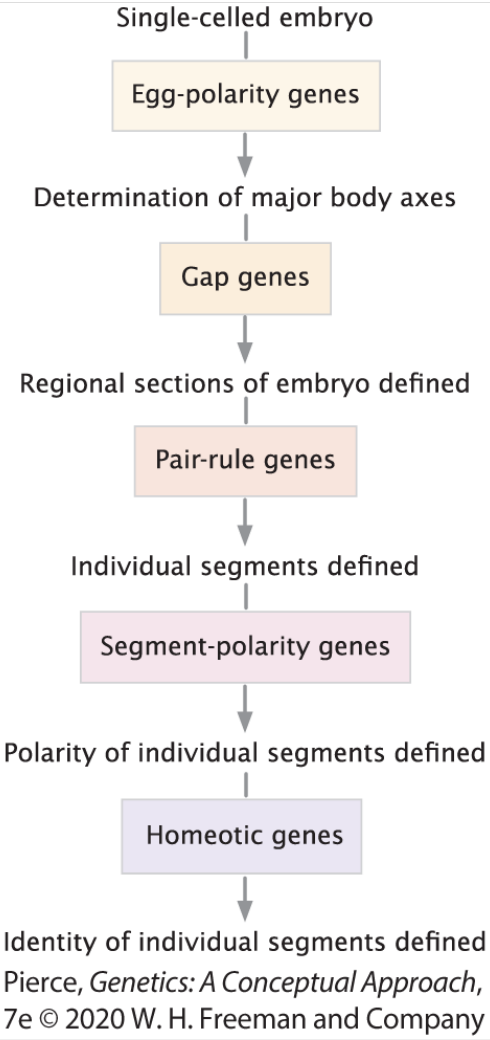
cluster, whereas some fishes, with much more complex body architecture, have as many as 48 *Hox* genes in seven clusters.

CONNECTING CONCEPTS

The Control of Development

Development is a complex process consisting of numerous events that must take place in a highly specific sequence. Studies of fruit flies and other organisms reveal that this process is regulated by a large number of genes. In *Drosophila*, the dorsal–ventral axis and the anterior–posterior axis are established by maternal genes; these genes encode mRNAs and proteins that are localized to specific regions within the egg and cause specific genes to be expressed in different regions of the embryo. The protein products of these genes then stimulate other genes, which in turn stimulate still other genes in a cascade of control. As might be expected, most of the gene products in the cascade are regulatory proteins, which bind to DNA and activate other genes.

In the course of development, successively smaller regions of the embryo are determined (**Figure 22.13**). In *Drosophila*, first the major axes and regions of the embryo are established by egg-polarity genes. Next, patterns within each region are determined by the action of segmentation genes: the gap genes define large sections of the embryo, the pair-rule genes define regional sections and affect alternate segments, and the segment-polarity genes affect individual segments. Finally, the homeotic genes provide each segment with a unique identity. Initial gradients in proteins and mRNA stimulate localized gene expression, which produces more finely located gradients that stimulate even more localized gene expression. Developmental regulation thus becomes more and more narrowly defined.



22.13 A cascade of gene regulation establishes the polarity and identity of individual segments of *Drosophila.* In development, successively smaller regions of the embryo are determined.

Description

In the beginning, there is a single-cell embryo. Egg-polarity genes are expressed, which determine the major body axes. Next, gap genes get expressed, which define the regional sections of embryo. Next, pair-rule genes become expressed, leading to the development of individual segments. Next, segment polarity genes are expressed, determining the polarity of individual segments. Then, homeotic genes are expressed, defining the identity of individual segments.

The processes by which limbs, organs, and tissues form (called morphogenesis) are less well understood, although this pattern of generalized-to-localized gene expression is encountered frequently. › **TRY PROBLEM 22**

Epigenetic Changes in Development

As we have just seen, early development in the fruit fly is controlled in large part by the products of certain key genes selectively activating or repressing the expression of other genes. As discussed in Chapter 21, gene expression in eukaryotes is affected by epigenetic changes, and indeed, epigenetics also plays an important role in development.

In Chapter 21, epigenetic changes were defined as heritable alterations to DNA and chromatin structure—alterations that affect gene expression and are passed on to other cells but are not changes to the DNA base sequence. In the course of development, a single-celled zygote divides and gives rise to many cells, which differentiate and acquire the characteristics of specific organs and tissues. Each type of cell eventually expresses a different subset of genes, producing the proteins needed for that cell type, and this program of gene expression is passed on when the differentiated cell divides.

The gene-expression program of cells that make up a particular organ or tissue type is often defined by epigenetic marks. As

development and differentiation proceed, cells acquire epigenetic changes that turn specific sets of genes on and off. In Chapter 21, we considered several types of epigenetic marks, including DNA methylation, the modification of histone proteins, and changes due to small RNA molecules. These epigenetic changes help determine which genes are expressed by a cell. In early stages of development, genes that may be required at later stages are often held in a transiently silent state by histone modifications. Histone modifications are generally flexible and easily reversed, so these genes can be activated in later developmental stages. In the course of development, other genes are permanently silenced; this longer-term silencing is often accomplished by DNA methylation.

Mapping Gene Expression in *Drosophila* Embryos

Using advances that allow RNA sequencing of single cells (see Chapter 20), researchers have determined gene expression patterns for over 8000 genes in 6000 cells that make up early *Drosophila* embryos. This transcriptional information is providing important insights into the genetic control of early *Drosophila* development. For example, genes for some transcription factors and long noncoding RNA molecules (lncRNAs, see Chapter 14) that were never previously implicated in *Drosophila* development were identified as having patterns of gene expression associated with early development.

These genes and their products are now being studied in more detail by developmental geneticists.

22.3 Genes Control the Development of Flowers in Plants

We have now examined in detail pattern formation in *Drosophila*, which serves as a model system of development. Another model system that has provided important insight into how genes influence patterns of growth and development is the formation of flower parts in angiosperms.

One of the most important developmental events in the life of a plant is the switch from vegetative growth to flowering. The precise timing of this switch is affected by season, day length, plant size, and a number of other factors and is under the control of a large number of different genes. The development of the flower itself is also under genetic control, and homeotic genes play a crucial role in the determination of the floral structures.

Flower Anatomy

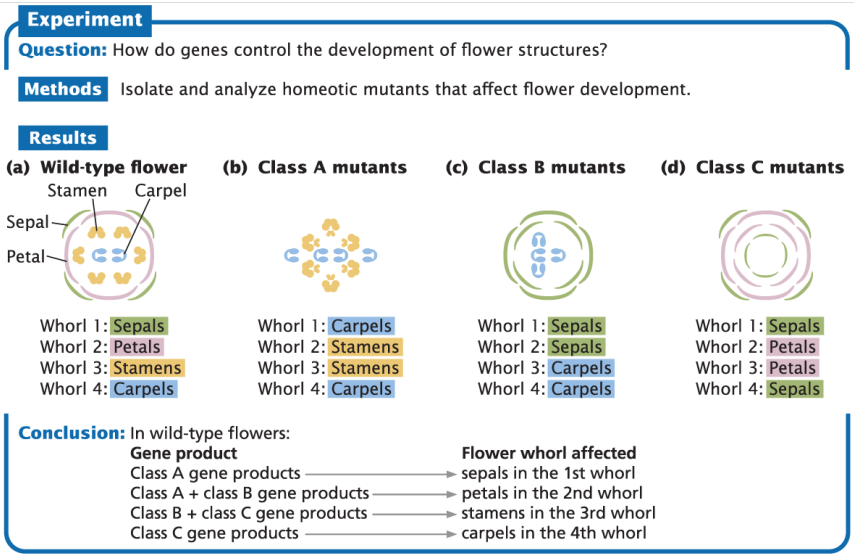
A flower is made up of four concentric rings of modified leaves, called whorls. The outermost whorl (whorl 1) consists of the green, leaflike sepals. The next whorl (whorl 2) consists of the petals, which typically lack chlorophyll. Whorl 3 consists of the stamens, which bear pollen, and whorl 4 consists of the carpels, which are often fused to form the stigma bearing the ovules. In wild-type *Arabidopsis*,

a model genetic plant (see the Reference Guide to Model Genetic Organisms and Figure 22.14), there are four sepals, four white petals, six stamens (four long and two short), and two carpels (Figure 22.15a).

THINK-PAIR-SHARE Question 4

Darwin Dale/Science Source.

22.14 The flower produced by *Arabidopsis thaliana* has four sepals, four white petals, six stamens, and two carpels.



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22.15 Analysis of homeotic mutants in *Arabidopsis thaliana* led to an understanding of the genes that determine floral structures in plants.

Description

The question in this experiment is: How do genes control the development of flower structures? The methods section reads, Isolate and analyze homeotic mutants that affect flower development. The results section shows four types of flowers. Mutant plants that had altered flower structure are examined. The whorls of each plant are represented as simple schematic drawings. Green lines are sepals, pink lines are petals, yellow circles are stamens, and blue horseshoes are carpels. Whorl 1 is the outer layer of the flower and whorl 4 is the inner layer of the flower. In wild-type flowers labeled “a”, whorls 1, 2, 3, and 4 have sepals, petals, stamens, and carpels, respectively. The mutant plants were categorized into 3 groups labeled “b” through “d.” Class A mutants labeled “b” developed carpels, stamen, stamen, carpels in whorls 1, 2, 3, 4, respectively. Class B mutants labeled “c” developed sepals, sepals, carpels, carpels in whorls 1, 2, 3, 4, respectively. Class c mutants labeled “d” developed sepals, petals, petals, sepals in whorls 1, 2, 3, 4, respectively. In conclusion, class A mutants are involved with producing sepals in whorl 1 and petals in whorl 2. Class b mutants are involved with producing petals in whorl 2 and stamens in whorl 3. Class c mutants are involved with producing stamens in whorl 3 and carpels in whorl 4.

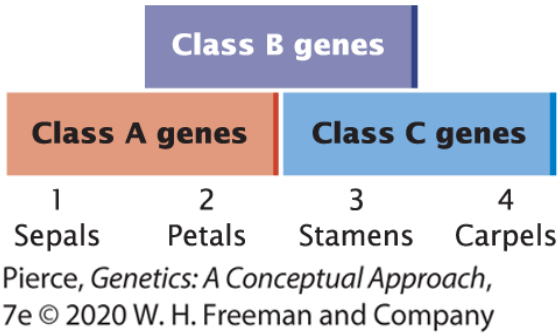
Genetic Control of Flower Development

Elliot Meyerowitz and his colleagues conducted a series of experiments in the late 1980s and early 1990s to examine the genetic basis of flower development in *Arabidopsis*. They began by isolating and analyzing homeotic mutants in *Arabidopsis*. Homeotic mutations were actually first identified in plants in 1894, when William Bateson noticed that the floral parts of plants occasionally appeared in the wrong place: he found, for example, flowers in which stamens grew in the place where petals normally grow.

Meyerowitz and his coworkers used these types of mutants to reveal the genes that control flower development. They were able to place the homeotic mutations that they isolated into three groups on the basis of their effect on floral structure. Class A mutants had carpels instead of sepals in the first whorl and stamens instead of petals in the second whorl (**Figure 22.15b**). The third whorl consisted of stamens, and the fourth whorl consisted of carpels, the normal pattern. Class B mutants had sepals in the first and second whorls and carpels in the third and fourth whorls (**Figure 22.15c**). The final group, class C mutants, had sepals and petals in the first and second whorls, respectively, as is normal, but had petals in the third whorl and sepals in the fourth whorl (**Figure 22.15d**).

Meyerowitz and his colleagues concluded that each class of mutants was missing the product of a gene, or the products of a set of genes, critical to proper flower development: class A mutants were missing gene A activity, class B mutants were missing gene B activity, and class C mutants were missing gene C activity. They hypothesized that the class A genes are active in the first and second whorls. Class A gene products alone cause the first whorl to differentiate into sepals, and together with class B gene products, they cause the second whorl to develop into petals. Class C gene products, together with class B gene products, induce the third whorl to develop into stamens. Class C genes alone cause the fourth whorl to become carpels. The products of the different gene classes and their effects are summarized in the conclusion of Figure 22.15.

To explain their results, they also proposed that the genes of some classes affect the activities of others. Where class A is active, class C is repressed, and where class C is active, class A is repressed. Additionally, if a mutation inactivates class A, then class C becomes active, and vice versa. Class A genes are normally expressed in whorls 1 and 2, class B genes are expressed in whorls 2 and 3, and class C genes are expressed in whorls 3 and 4 (**Figure 22.16**).



22.16 Expression of class A, B, and C genes varies among the structures of a flower.

Description
Below boxes A and C show whorl numbers 1 through 4 as follows: Below class A box, 1 sepals and 2 petals; below class C box, 3 stamens and 4 carpels.

The interaction of these three classes of genes explains the different classes of mutants in Figure 22.15. For example, class A mutants are lacking class A gene products, and therefore class C genes are active in all tissues, because when A is inactivated, C becomes active. Therefore, whorl 1, with only class C gene products, will consist of carpels; whorl 2, with class C and class B gene products, will produce stamens; whorl 3, with class B and class C gene products, will produce stamens; and whorl 4, with only class C gene activity, will produce carpels (see Figure 22.15b):

Class C (in the absence of class A) gene products	→ carpels (1st whorl)
Class B + class C (in the absence of class A) gene products	→ stamens (2nd whorl)
Class B + class C gene products	→ stamens (3rd whorl)
Class C gene products	→ carpels (4th whorl)

To confirm this explanation, Meyerowitz and his colleagues bred double and triple mutants and predicted the outcome. The resulting flower structures fit their predictions. In subsequent studies, they isolated the genes of each class. There are two class A genes, termed *APETALA1* (*AP1*) and *APETALA2* (*AP2*); two class B genes, termed *APETALA3* (*AP3*) and *PISTILLATA* (*PI*); and one class C gene, termed *AGAMOUS* (*AG*). The cloning and sequencing of these genes revealed that all are MADS-box genes—genes whose products function as transcription factors and affect the expression of other genes. MADS-box genes in plants play a role similar to that of homeobox genes in animals, although MADS-box genes and homeobox genes are not homologous.

The results of other studies have demonstrated the presence of an additional group of genes, called *SEPALLATA* (*SEP*), that are expressed in whorls 2, 3, and 4, and they, too, are required for normal floral development. If the *SEP* genes are defective, the flower consists entirely of sepals. Findings from studies of other species have demonstrated that this system of flower development exists not only in *Arabidopsis* but also in other flowering plants. It is important to note that these genes are necessary but not sufficient for proper flower development; other genes also take part in determining the identity of the different parts of flowers. › **TRY PROBLEM 25**

CONCEPTS

Homeotic genes control the development of floral structures in plants. The products of homeotic genes interact to determine the formation of the four whorls that constitute a complete flower.

✔ CONCEPT CHECK 5

What types of flower structures would you expect to see in whorls 1 through 4 of a mutant plant that failed to produce both class A and class B gene products?

a. Carpels, stamens, stamens, carpels
b. Sepals, sepals, carpels, carpels
c. Sepals, sepals, sepals, sepals
d. Carpels, carpels, carpels, carpels

CONNECTING CONCEPTS CONNECT

Comparison of Development in *Drosophila* and Flowers

We have now considered two very different model systems of development: the development of body form and pattern in fruit flies and the development of flower structures in angiosperms. In spite of their differences, these two systems exhibit similarities in how genes control development.

First, both pattern formation in *Drosophila* and flower development in plants are controlled by numerous genes that interact in complex ways. For example, we saw in *Drosophila* how a large complex of genes successively defines smaller and smaller regions of the fruit-fly embryo and how genes at one level stimulate and inhibit genes at other levels. Similarly, flower development is controlled by class A, class B, and class C genes. The action of each class depends on which products of other classes are present, and the individual genes of each class interact in complex ways to control the differentiation of each whorl of a flower.

Another common feature of development in fruit flies and in flowers is that many of the genes involved in these processes function by influencing the expression of other genes. In both fruit flies and flowers, there is a cascade of development in which gene products stimulate other genes, which in turn stimulate still other genes. Many of the gene products are regulatory proteins that bind to DNA and affect the transcription of other genes. For example, *Hox* genes

in *Drosophila* and MADS-box genes in flowers encode transcription factors that play an important role in development.

A final similarity is that each system contains homeotic genes, which define the identity of particular structures or segments. Mutation of these homeotic genes produces structures that are in the wrong place, such as legs where antennae are normally found in fruit flies or carpels where sepals usually occur in flowers.

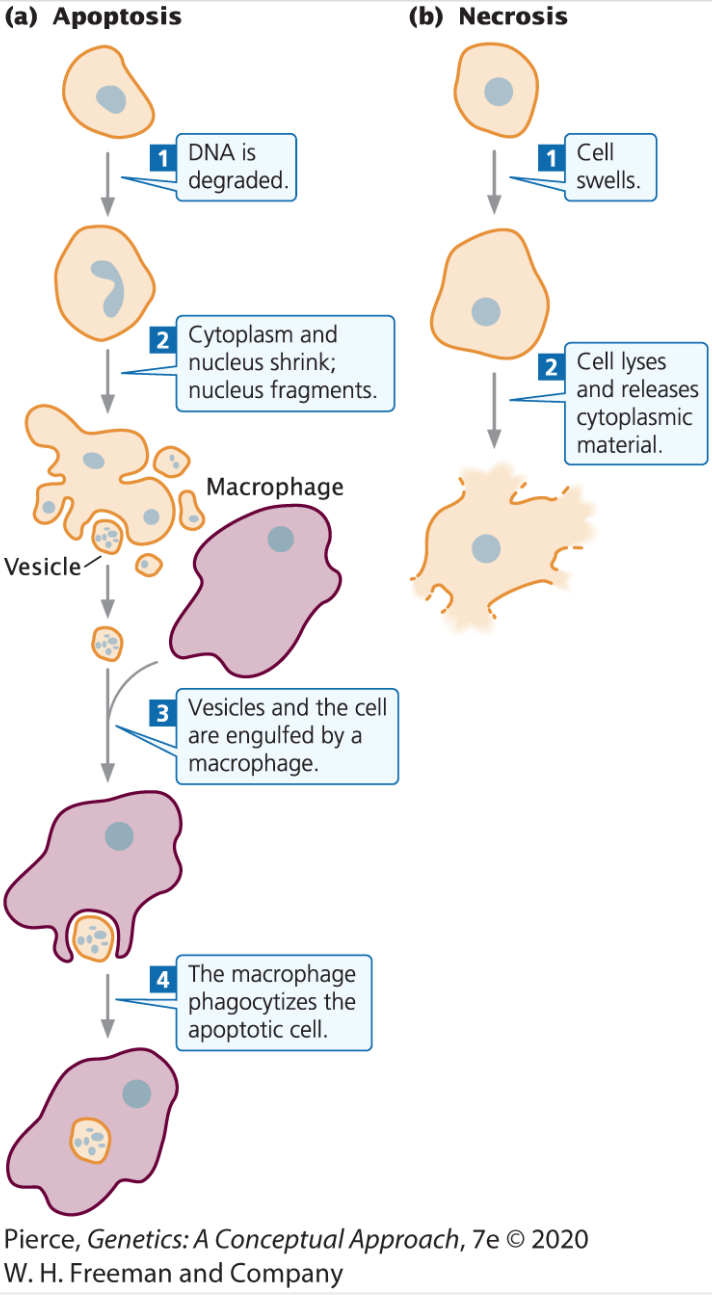
22.4 Programmed Cell Death Is an Integral Part of Development

An important aspect of development is the death of cells. Cell death shapes many body parts in the course of development: it is responsible for the disappearance of a tadpole's tail during metamorphosis and causes the removal of tissue between the digits to produce the human hand. Cell death is also used to eliminate dangerous cells that have escaped normal controls (see the section on mutations in cell cycle control genes and cancer in Chapter 23).

APOPTOSIS

Cell death in animals is often initiated by the cell itself in a process called **apoptosis**, or programmed cell death. In this process, a cell's DNA is degraded, its nucleus and cytoplasm shrink, and blebbing occurs: the cell membrane bulges outward and breaks off into vesicles, taking some cytoplasm with it. These vesicles, and the cell itself, undergo phagocytosis by other cells without any leakage of cellular contents or damage to nearby cells (**Figure 22.17a**). Cells that are injured, on the other hand, die by a relatively uncontrolled process called *necrosis*. In this process, a cell swells and bursts, spilling its contents over neighboring cells and eliciting an inflammatory response (**Figure 22.17b**). Apoptosis is essential to

embryogenesis; most multicellular animals cannot complete development if the process is inhibited.



22.17 Programmed cell death by apoptosis is distinct from uncontrolled cell death through necrosis.

Description
Part “a” labeled apoptosis starts with a cell with D N A. A caption reads, “D N A is degraded.” The cell then shows degraded D N A. A caption reads, “Cytoplasm and nucleus shrink; nucleus fragments.” Cytoplasm shows degradation with scattered D N A. A macrophage is shown approaching the cell. A caption reads, “Vesicles and the cell are engulfed by macrophage.” The macrophage engulfs the cell through pseudopodias. A caption reads, “Macrophage phagocytizes apoptotic cell.” The macrophage then shows the cell inside it. Part “b” labeled necrosis starts with a similar cell having D N A. A caption reads, “Cell swells.” The cytoplasm shows an abnormal shape and then lysis of cytoplasm occurs. A caption reads, “Cell lyses and releases cytoplasmic material.”

REGULATION OF APOPTOSIS

Surprisingly, most cells are programmed to undergo apoptosis and will survive only if the internal death program is continuously held in check. Apoptosis is highly regulated and depends on numerous signals from inside and outside the cell. Geneticists have identified a number of genes that have roles in various stages of the regulation of apoptosis. Some of these genes encode enzymes called **caspases**, which cleave other proteins at specific sites (after aspartic acid). Each caspase is synthesized as a large, inactive precursor (a procaspase) that is activated by cleavage, often by another caspase. When one caspase is activated, it cleaves other procaspases, which trigger even more caspase activity. The resulting cascade of caspase activity eventually cleaves proteins essential to cell function, such as those supporting the nuclear membrane and cytoskeleton. Caspases also cleave a protein that normally keeps an enzyme that degrades DNA (DNase) in an inactive form. Cleavage of this protein activates

DNase and leads to the breakdown of cellular DNA, which eventually leads to cell death.

Procaspases and other proteins required for cell death are continuously produced by healthy cells, so the potential for cell suicide is always present. A number of different signals can trigger apoptosis; for instance, infection by a virus can activate immune-system cells to secrete substances onto an infected cell, causing that cell to undergo apoptosis. This process is believed to be a defense mechanism designed to prevent the reproduction and spread of viruses. Similarly, DNA damage can induce apoptosis and thus prevent the replication of mutated sequences. Damage to mitochondria or the accumulation of a misfolded protein in the endoplasmic reticulum also stimulate programmed cell death.

In addition to factors that induce apoptosis, there are other proteins called survival factors that prevent inappropriate or premature cell death by actively suppressing the process of apoptosis. Survival factors often act by inhibiting caspases and other proteins involved in apoptosis. Programmed cell death is thus regulated by the balance between pro-apoptosis factors that stimulate apoptosis and survival factors that hold it in check.

APOPTOSIS IN DEVELOPMENT

Apoptosis plays a critical role in development. As animals develop, excess cells are often produced and then later culled by apoptosis to

produce the proper number of cells required for an organ or a tissue. In some cases, whole structures are created that are later removed by apoptosis. For example, early mammalian embryos develop both male and female reproductive ducts, but the Wolffian ducts degenerate in females and the Mullerian ducts degenerate in males. Apoptosis also plays an important role in the development of immunity (see Section 22.6), in which lymphocytes that recognize the body's own cells normally undergo apoptosis so that they do not attack self-tissues.

During embryonic development in *Drosophila*, large numbers of cells die. Three genes in *Drosophila* activate caspases that are essential for apoptosis: *reaper* (*rpr*), *grim*, and *head involution defective* (*hid*). Embryos possessing a deletion that removes all three genes exhibit no apoptosis and die in the course of embryogenesis with an excess of cells. Numerous other genes also affect the process of apoptosis.

Apoptosis is also crucial in metamorphosis, the process by which larval structures are transformed into adult structures. For example, the large salivary glands of larval fruit flies regress during metamorphosis. The hormone ecdysone stimulates metamorphosis, including the onset of apoptosis. Ecdysone induces the expression of *rpr* and *hid* and inhibits the expression of other genes, which then leads to apoptosis of salivary gland cells.

THINK-PAIR-SHARE Question 5

CONCEPTS

Cells are capable of apoptosis (programmed cell death), a highly regulated process that depends on enzymes called caspases. Apoptosis plays an important role in animal development.

✔ CONCEPT CHECK 6

How does cell death from apoptosis differ from cell death from necrosis?

22.5 The Study of Development Reveals Patterns and Processes of Evolution

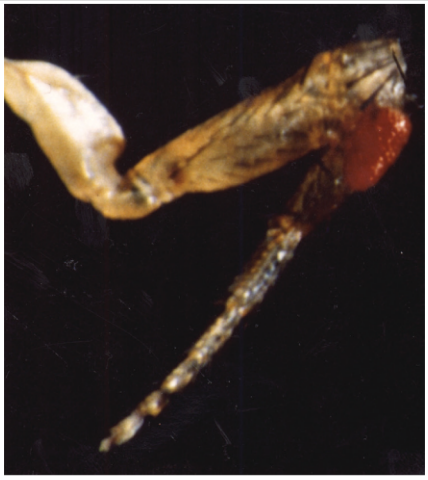
"Ontogeny recapitulates phylogeny" is a familiar phrase that was coined in the 1860s by German zoologist Ernst Haeckel to describe his belief—now considered an oversimplification—that during their development (ontogeny), organisms repeat their evolutionary history (phylogeny). According to Haeckel's belief, a human embryo passes through fish, amphibian, reptilian, and mammalian stages before developing human traits. Scientists have long recognized that organisms do not pass through the adult stages of their ancestors during their development, but the embryos of these related organisms often display similarities.

COMMON GENES IN DEVELOPMENTAL PATHWAYS

Although ontogeny does not precisely recapitulate phylogeny, many evolutionary biologists today are turning to the study of development for a better understanding of the processes and patterns of evolution. Sometimes called "evo-devo," the study of evolution through the analysis of development is revealing that the same genes often shape developmental pathways in distantly related organisms. Biologists once thought that segmentation in vertebrates and invertebrates was only superficially similar, but we

now know that in both *Drosophila* and the primitive chordate *Branchiostoma*, the *engrailed* gene divides the embryo into specific segments. A gene called *Distal-less*, which creates the legs of a fruit fly, plays a role in the development of crustacean branched appendages. This same gene stimulates body outgrowths in many other organisms, from polychaete worms to starfish. Another example is *Pitx1*, whose role in controlling the presence and absence of pelvic spines in sticklebacks was discussed in the introduction to this chapter. This same gene is found in mice, where it also affects hind limb development, and in humans, where mutations of the gene have been associated with the development of club foot.

An amazing example of how the same genes in distantly related organisms can shape similar developmental pathways is seen in the development of eyes in fruit flies, mice, and humans. Walter Gehring and his collaborators examined the effect of the *eyeless* gene in *Drosophila*, which is required for proper development of the fruit-fly eye. Gehring and his coworkers genetically engineered cells that expressed *eyeless* in parts of the fly where the gene is not normally expressed. When these flies hatched, they had eyes on their wings, antennae, and legs (**Figure 22.18**). These structures were not just tissue that resembled eyes but complete eyes, with a cornea, cone cells, and photoreceptors that responded to light, although the flies could not use these eyes to see because they lacked a connection to the nervous system.



U. Kloter & G. Halder/VIB Center for the Biology of Disease, KU Leuven.

22.18 Expression of the *eyeless* gene causes the development of an eye on the leg of a fruit fly. Genes similar to *eyeless* also control eye development in mice and humans.

The *eyeless* gene has counterparts in mice and humans that affect the development of mammalian eyes. There is a striking similarity between the *eyeless* gene of *Drosophila* and the *Small eye* gene that exists in mice. In mice, a mutation in one copy of *Small eye* causes small eyes; a mouse that is homozygous for the *Small eye* mutation has no eyes. There is also a similarity between the *eyeless* gene in *Drosophila* and the *Aniridia* gene in humans; a mutation in *Aniridia* produces a severely malformed human eye. Similarities in the sequences of *eyeless*, *Small eye*, and *Aniridia* suggest that all three genes evolved from a common ancestral sequence and that a common pathway underlies eye development in flies, mice, and humans. This possibility is surprising because the eyes of insects and mammals were thought to have evolved independently.

Similar genes may be part of a developmental pathway common to two different species but have quite different effects. For example, a *Hox* gene called *Abdominal B* (*AbdB*) helps define the posterior end of a *Drosophila* embryo; a similar group of genes in birds divides the wing into three segments. In another example, the *short gastrulation* (*sog*) gene in fruit flies stimulates cells to assume a ventral orientation in the embryo, but the expression of a similar gene called *chordin* in vertebrates causes cells to assume a dorsal orientation, exactly the opposite of the situation in fruit flies. In vertebrates, *toll* genes encode proteins called Toll-like receptors, which bind to molecules on pathogens and stimulate the immune system. In fruit flies, the *toll* gene similarly functions in immunity, but it also encodes a protein that helps determine the dorsal–ventral axis, as mentioned in Section 22.2. The theme emerging from these studies is that a small shared set of genes may underlie many basic developmental processes in many different organisms.

EVOLUTION THROUGH CHANGE IN GENE EXPRESSION

Another principle revealed by studies in evo-devo is that many major evolutionary adaptations are accomplished not through changes in the types of proteins produced but through changes in the expression of genes that encode proteins that regulate development. This principle is illustrated in the introduction to this chapter, where we saw that deletion of an enhancer that stimulates

the *Pitx1* gene in sticklebacks is responsible for the evolution of pelvic spine reduction in freshwater populations of the fish.

This principle is also seen in the evolution of blind cavefish. The Mexican tetra, *Astyanax mexicanus*, normally occurs in surface waters of streams and rivers in Texas and northern Mexico. Some 10,000 years ago, a few tetras migrated into caves. In the total darkness of their cave environment, vision was not needed, and with the passage of time, these tetras lost their eyes. Today, some 30 distinct populations of Mexican tetras are totally blind and eyeless (**Figure 22.19**), whereas surface-dwelling populations of the same species have retained normal eye development.

Mark Smith/Science Source.

22.19 Mexican tetras that live in caves have lost their eyes through a developmental change in gene expression.

How did Mexican cave-dwelling tetras lose their eyes? Blind Mexican tetra zygotes begin to develop eyes, just like their surface-dwelling cousins, but about 24 hours after fertilization, eye development is aborted, and the cells that were destined to become the lens spontaneously die. The absence of the lens prevents other

components of the eye, such as the cornea and iris, from developing. The optic cup and retina form, but their growth is retarded, and photoreceptor cells never differentiate. The degenerate eye sinks into the orbit and is eventually covered by a flap of skin.

Blind Mexican tetras have the same genes as surface-dwelling Mexican tetras; how they differ is in gene expression. Two genes, named *sonic hedgehog* (*shh*) and *tiggy-winkle hedgehog* (*twhh*), are more widely expressed in the eye primordium of blind cavefish than in surface fish. (The original *hedgehog* gene was named for a mutant phenotype in *Drosophila*, in which the mutant embryo is covered with denticles, like a hedgehog. The *sonic hedgehog* gene is named after the video game character, and the *tiggy-winkle hedgehog* gene is named for Mrs. Tiggy-Winkle, a character from Beatrix Potter's books.) The expanded expression of *shh* and *twhh* activates the transcription of other genes, which cause lens cells to undergo apoptosis, and the lens degenerates.

When geneticists injected *twhh* or *shh* mRNA into the embryos of surface fish, the development of the lens was aborted, and adults that developed from these embryos were missing eyes. When drugs were used to inhibit the expression of *twhh* and *shh* in cavefish embryos, eye development in these fish was partly restored. These results demonstrate that eye development in Mexican tetras is regulated by the precise expression of *shh* and *twhh*.

Overexpression of one or both of these genes in the cavefish induces the death of lens cells and aborts normal eye development. A small increase in the transcription of either gene during embryonic development results in a major anatomical change in the adult fish, a change that has allowed these fish to adapt to the darkness of the cave environment.

Other research demonstrates that eye degeneration of blind Mexican tetras is affected by epigenetic modifications (see Chapter 21 for a discussion of epigenetic modifications). One type of epigenetic modification is DNA methylation, in which methyl groups are added to cytosine bases by methyltransferase enzymes. DNA methylation generally represses transcription. Researchers determined that eye degeneration in blind Mexican tetras is preceded by decreased transcription of a number of genes involved in eye development. In blind cavefish, expression of the *dnmt3bb.1* gene, which encodes a methyltransferase enzyme, is increased 1.5 times compared to surface fish with eyes. The excessive expression of the *dnmt3bb.1* gene in the blind fish leads to greater levels of DNA methylation, which then represses transcription of other genes involved in eye development. Researchers demonstrated the importance of DNA methylation in eye development of these fish by injecting 5-Azacytidine (AZA), an inhibitor of DNA methylation, into one eye of some developing blind cavefish embryos and injecting a control substance into one eye of other fish embryos. Five days later, eyes that were injected with AZA were significantly larger than

uninjected eyes on the same animal and larger than eyes of fish injected with the control substance. These results indicate that DNA methylation plays an important role in eye degeneration of blind Mexican tetras. ❯ **TRY PROBLEM 29**

Another example of differences in gene expression bringing about evolutionary change is seen in Darwin's finches, a group of closely related bird species found on the Galápagos Islands (see Figure 26.6). The species differ primarily in the size and shape of their beaks: ground finches have deep and wide beaks, cactus finches have long and pointed beaks, and warbler finches have sharp and thin beaks. These differences are associated with diet, and evolutionary changes in beak shape and size have taken place in the past, when climate changes brought about shifts in the abundance of food items.

To investigate the underlying genetic basis of these evolutionary changes, Arkaht Abzhanov and his colleagues used microarrays (see Section 20.2) to examine differences in the transcription levels of several thousand genes in five species of Darwin's finches. They found differences in the expression of a gene that encodes a protein called calmodulin (CaM); the gene that encodes CaM was more highly expressed in the long and pointed beaks of cactus-finch embryos than in the beaks of the other species. CaM takes part in a process called calcium signaling, which is known to affect many aspects of development. When Abzhanov and his coworkers activated calcium signaling in developing chicken embryos, the

chickens had longer beaks, resembling those of the cactus finch. Thus, these researchers were able to reproduce, at least in part, the evolutionary difference that distinguishes cactus finches. This experiment shows that changes in the expression of a single gene in the course of development can produce significant anatomical differences in adults.

In these studies and others, the combined efforts of developmental biologists, geneticists, and evolutionary biologists are providing important insights into how evolution takes place. Although Haeckel's euphonious phrase "ontogeny recapitulates phylogeny" was incorrect, evo-devo is proving that development can reveal much about the process of evolution.

22.6 The Development of Immunity Occurs Through Genetic Rearrangement

As we have seen in our consideration of animal and plant development, a basic principle of developmental biology is that every somatic cell carries an identical set of genetic information, and no genes are lost in development. Although this principle holds for most cells, there are some important exceptions, one of which concerns genes that encode immune function in vertebrates. In the development of immunity, individual segments of certain genes are rearranged into different combinations, producing immune-system cells that contain different genetic information and are each adapted to attack one particular foreign substance. This rearrangement and loss of genetic material is key to the power of our immune systems to protect us against almost any conceivable foreign substance.

The immune system provides protection against infection by bacteria, viruses, fungi, and parasites. The focus of an immune response is an **antigen**, defined as any molecule (usually a protein) that elicits an immune reaction. The immune system is remarkable in its ability to recognize an almost unlimited number of potential antigens. The body is full of proteins, so it is essential that the immune system be able to distinguish between self-antigens and foreign antigens. Occasionally, the ability to make this distinction

breaks down, and the body produces an immune reaction to its own antigens, resulting in an **autoimmune disease** (**Table 22.5**).

TABLE 22.5 Examples of autoimmune diseases

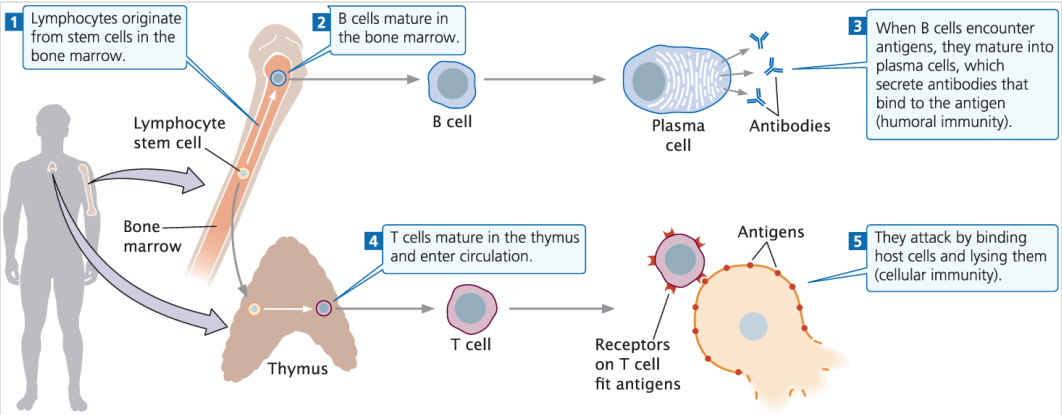
Disease	Tissues Attacked
Graves disease, Hashimoto thyroiditis	Thyroid gland
Rheumatic fever	Heart muscle
Systemic lupus erythematosus	Joints, skin, and other organs
Rheumatoid arthritis	Joints
Insulin-dependent diabetes mellitus	Insulin-producing cells in pancreas
Multiple sclerosis	Myelin sheath around nerve cells

The Organization of the Immune System

The immune system contains a number of different components and uses several mechanisms to provide protection against pathogens, but most immune responses can be grouped into two major classes: humoral immunity and cellular immunity. Although it is convenient to think of these classes as separate systems, they interact with and influence each other significantly.

HUMORAL IMMUNITY

Immune function is carried out by specialized blood cells called lymphocytes, which are a type of white blood cell. **Humoral immunity** centers on the production of antibodies by specialized lymphocytes called **B cells** (**Figure 22.20**), which mature in the bone marrow. **Antibodies** are proteins that circulate in the blood and other body fluids, where they bind to specific antigens and mark them for destruction by phagocytic cells. Antibodies also activate a set of proteins called *complement*, which help to lyse cells and attract macrophages.



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22.20 Immune responses are classified as humoral immunity, in which antibodies are produced by B cells, and cellular immunity, which is produced by T cells.

Description
The diagram starts with a human outline, which leads to two branches. One branch shows a diagram of a bone marrow with a lymphocyte stem cell that develops into a B cell. Two captions read, “Lymphocytes originate from stem cells in the bone marrow” and “B cells mature in the bone marrow.” The B cell then develops into a plasma cell which then gives rise to Y-shaped antibodies. Another caption reads, “When B cells encounter antigens, they mature into plasma cells, which secrete antibodies that bind to the antigen (humoral immunity).” The second branch shows the thymus. An arrow from lymphocyte stem cell inside the bone marrow leads to a cell in the thymus. This cell matures inside the thymus. It shows a T-cell. A caption reads, “T cells mature in the thymus and enter circulation.” The T-cell then shows receptors on its surface that fit antigens and is then showed attached to an antigen. Another caption reads, “They attack by binding host cells and lysing them (cellular immunity).”

CELLULAR IMMUNITY

T cells (see Figure 22.20) are specialized lymphocytes that mature in the thymus and respond only to antigens found on the surfaces of the body’s own cells. These lymphocytes are responsible for the second type of immune response, **cellular immunity**.

After a pathogen such as a virus has infected a host cell, some viral antigens appear on the cell’s surface. Proteins called **T-cell receptors** on the surfaces of T cells bind to these antigens and mark the infected cell for destruction. T-cell receptors must simultaneously

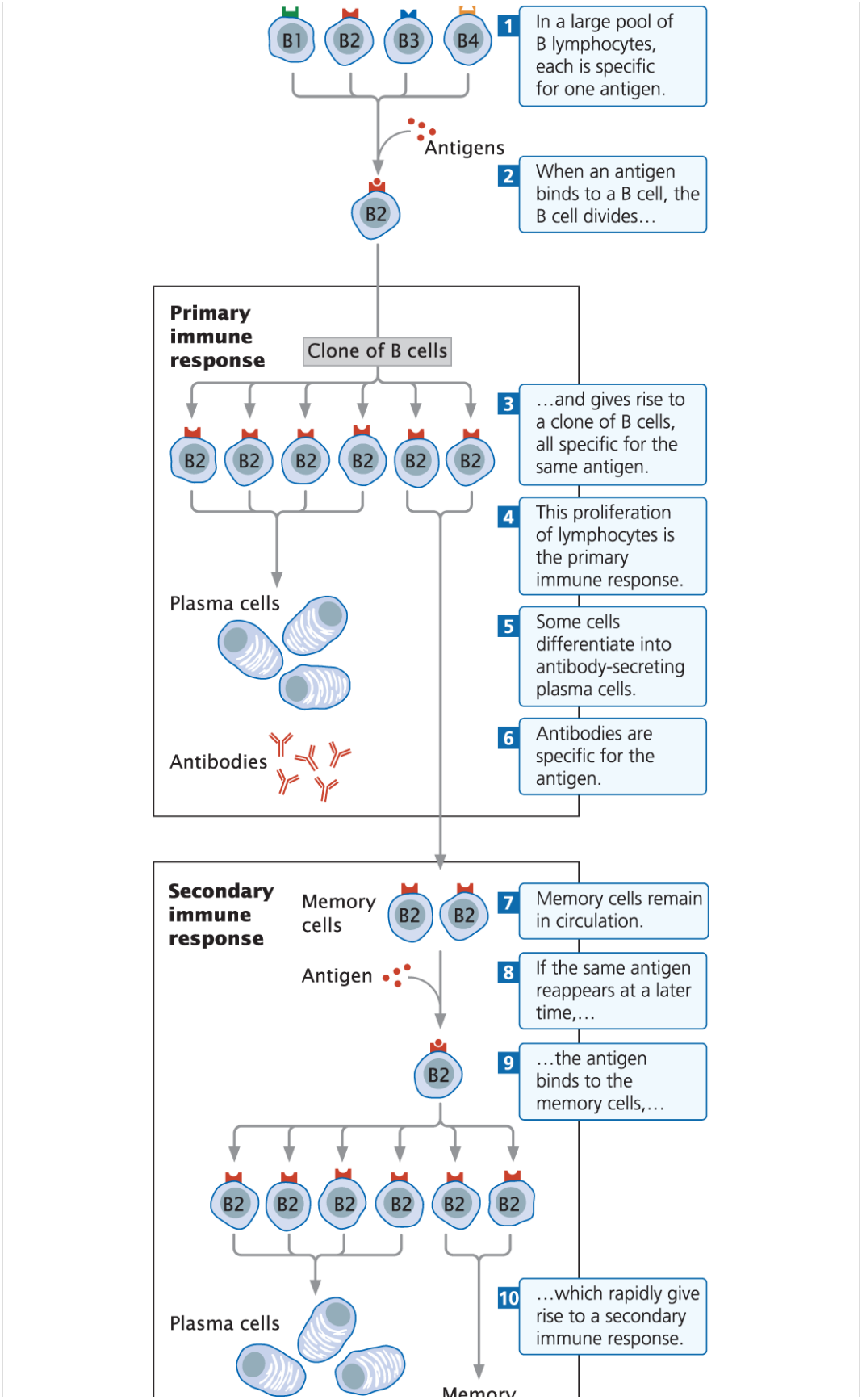
bind a foreign antigen and a self-antigen called a **major histocompatibility complex (MHC) antigen** on the host-cell surface (discussed later in this section). Not all T cells attack cells having foreign antigens; some help regulate immune responses, providing communication among different components of the immune system.

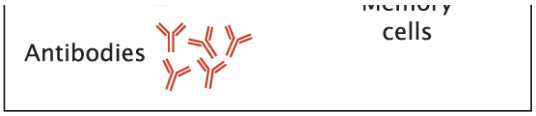
CLONAL SELECTION

How can the immune system recognize an almost unlimited number of foreign antigens? Remarkably, each mature lymphocyte is genetically programmed to attack one and only one specific antigen: each mature B cell produces antibodies against a single antigen, and each T cell is capable of attaching to only one type of foreign antigen.

If each lymphocyte is specific for only one type of antigen, how does an immune response develop? The **theory of clonal selection** states that initially, there is a large pool of millions of different lymphocytes, each capable of binding to only one antigen (**Figure 22.21**), so that millions of different foreign antigens can be detected. To illustrate clonal selection, let's imagine that a foreign protein enters the body. Only a few lymphocytes in the pool will be specific for this particular foreign antigen. When one of these lymphocytes encounters the foreign antigen and binds to it, that lymphocyte is stimulated to divide. The lymphocyte proliferates rapidly, producing

a large population of genetically identical cells—a clone—each of which is specific for that particular antigen.


B1
B2
B3
B4
1 In a large pool of B lymphocytes, each is specific for one antigen.
Antigens
2 When an antigen binds to a B cell, the B cell divides…
Primary immune response
Clone of B cells
3 …and gives rise to a clone of B cells, all specific for the same antigen.
4 This proliferation of lymphocytes is the primary immune response.
Plasma cells
5 Some cells differentiate into antibody-secreting plasma cells.
Antibodies
6 Antibodies are specific for the antigen.
Secondary immune response
Memory cells
7 Memory cells remain in circulation.
Antigen
8 If the same antigen reappears at a later time,…
9 …the antigen binds to the memory cells,…
Plasma cells
10 …which rapidly give rise to a secondary immune response.
Memory



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22.21 An immune response to a specific antigen is produced through clonal selection.

Description

The illustration starts with four B-cells labeled B-1 through B-4. A caption reads, “In a large pool of B lymphocytes, each is specific for one antigen.” Antigens are then shown binding to B-2. A caption reads, “When an antigen binds to a B cell, the B cell divides and gives rise to clones of the B cells, all specific for the same antigen.” This cell leads to primary immune response, in which the clones of the B-cells are shown. Six B-2 cells are shown; four of which lead to plasma cells and two lead to memory cells. The plasma cells produce Y-shaped antibodies. Three captions read as follows: “This proliferation of lymphocytes is the primary immune response. Some cells differentiate into antibody-secreting plasma cells. Antibodies are specific for the antigen.” The two B-2 cells known as memory cells lead to secondary immune response. A caption reads, “Memory cells remain in circulation.” Further, antigens bind to B-2 cells and the same process occurs. A caption reads, “If the same antigen reappears at a later time, the antigen binds to the memory cells, which rapidly give rise to a secondary immune response.”

This initial proliferation of antigen-specific B and T cells is known as a **primary immune response** (see Figure 22.21); in most cases, the primary response destroys the foreign antigen. Following the primary immune response, most of the lymphocytes in the clone die, but a few continue to circulate in the body. These **memory cells** may remain in circulation for years, or even for the rest of a person’s life. Should the same antigen reappear at some time in the future, memory cells specific to that antigen become activated and quickly give rise to another clone of cells capable of binding to the antigen.

The rise of this second clone is termed a **secondary immune response** (see Figure 22.21). This ability to quickly produce a second clone of antigen-specific cells permits the long-lasting immunity that often follows recovery from a disease. For example, people who have chicken pox usually have life-long immunity to the disease. The secondary immune response is also the basis for vaccination, which stimulates a primary immune response to an antigen and results in memory cells that can quickly produce a secondary response if that same antigen appears in the future.

Three sets of proteins are used in immune responses: antibodies, T-cell receptors, and the major histocompatibility antigens. The next section explores how the enormous diversity in these proteins is generated.

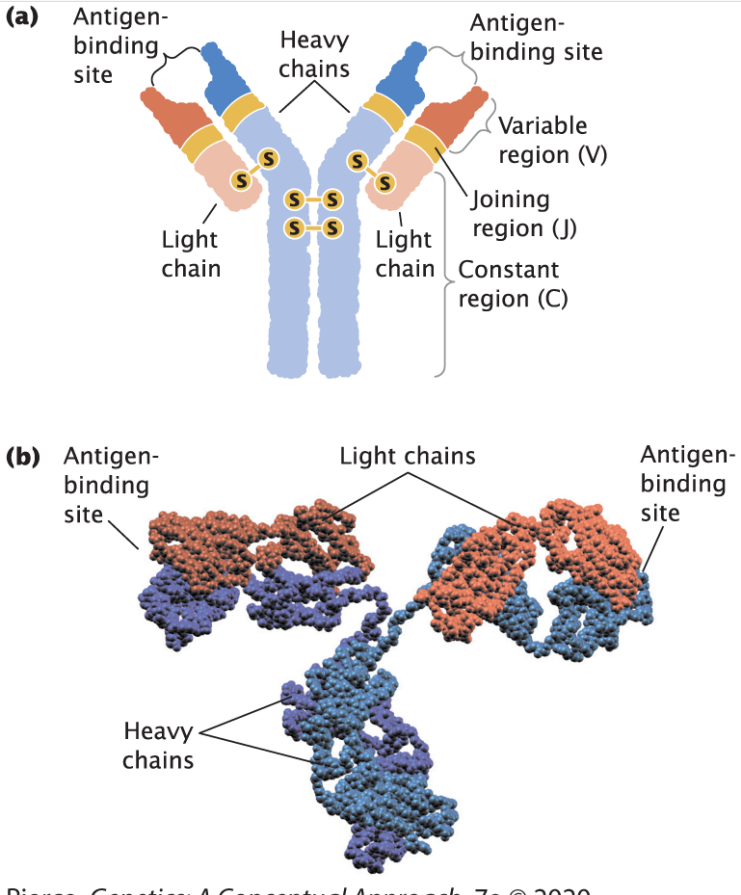
THINK-PAIR-SHARE Question 6

CONCEPTS

Each B cell and T cell of the immune system is genetically capable of binding to one type of foreign antigen. When a lymphocyte binds to an antigen, the lymphocyte undergoes repeated division, giving rise to a clone of genetically identical lymphocytes (the primary immune response), all of which are specific for that same antigen. Memory cells remain in circulation for long periods; if the antigen reappears, the memory cells rapidly proliferate and generate a secondary immune response.

Immunoglobulin Structure

The principal products of the humoral immune response are antibodies—also called immunoglobulins. Each immunoglobulin (Ig) molecule consists of four polypeptide chains—two identical light chains and two identical heavy chains—that form a Y-shaped structure (Figure 22.22). Disulfide bonds link the two heavy chains in the stem of the Y and attach a light chain to a heavy chain in each arm of the Y. Binding sites for antigens are at the ends of the two arms.



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22.22 Each immunoglobulin molecule consists of four polypeptide chains—two light chains and two heavy chains—that combine to form a Y-shaped structure. (a) Structure of an immunoglobulin. (b) Folded, space-filling model.

Description
Diagram “a” shows the heavy chains forming a Y-shaped structure. 2 disulfide bridges hold the Y together at its neck. 2 light chains attach to the heavy chain at the arms of the Y, and are held in place by one disulfide bridge. The bottom region is labeled constant. The middle of the arms of the Y are labeled joining region. The area above the joining region is labeled the variable region. The very top ends of the arms of the Y are labeled the antigen-binding site. Part “b” shows a space filling model of an immunoglobulin. The model shows a Y-shaped structure with heavy chains in blue color and light chains in red. Antigen binding sites are labeled at both top ends.

The light chains of an immunoglobulin are of two basic types: kappa chains and lambda chains. An immunoglobulin molecule can have two kappa chains or two lambda chains, but it cannot have one of each type. Both the light and the heavy chains have a variable region at one end and a constant region at the other end; the variable regions of different immunoglobulin molecules vary in their amino acid sequence, whereas the constant regions of different immunoglobulins are similar in sequence. The variable regions of both light and heavy chains make up the antigen-binding regions and specify the type of antigen to which the antibody can bind. The constant regions of the heavy chains at the base of the Y-shaped antibody help to determine the function of the antibody and its ability to communicate with other parts of the immune system. For example, these constant regions are important in the uptake and destruction of antigen-bound antibodies by phagocytic cells.

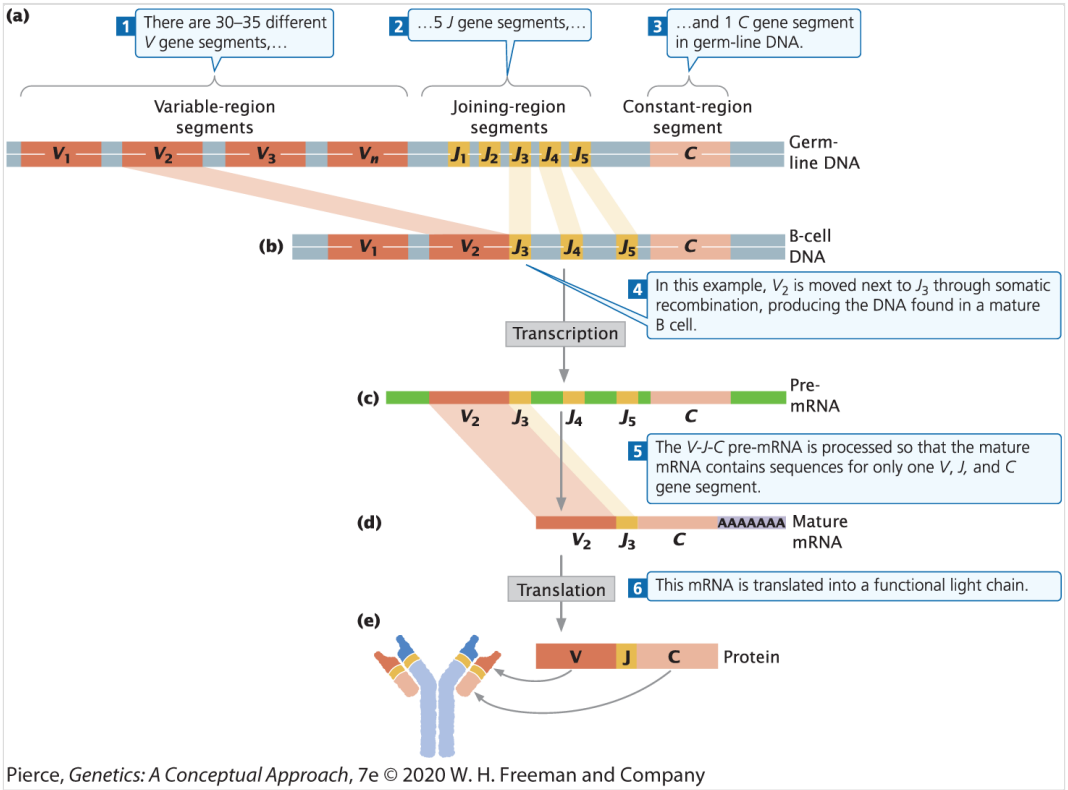
The Generation of Antibody Diversity

The human immune system is capable of making antibodies against virtually any antigen that might be encountered in a person's lifetime: each person is capable of making at least 10^{11} different antibody molecules. Antibodies are proteins, so the amino acid sequences of all 10^{11} potential antibodies must be encoded in the human genome. However, there are fewer than 1×10^5 genes in the human genome and, in fact, only about 3×10^9 total base pairs—so how can this huge diversity of antibodies be encoded?

The answer lies in the fact that antibody genes are composed of several distinct types of segments. There are a number of copies of each type of segment, each differing slightly from the others. In the maturation of a lymphocyte, segments of each type are joined together to create an immunoglobulin gene. The particular copy of each segment used is random, and because there are multiple copies of each type, there are many possible combinations of the segments. A limited number of segments can therefore encode a huge diversity of antibodies.

To illustrate this process of antibody assembly, let's consider the immunoglobulin light chains. Kappa and lambda chains are encoded by separate genes on different chromosomes. Each of these genes is composed of three types of segments: *V*, for variable; *J*, for joining; and *C*, for constant. The *V* segments encode most of the variable

region of a light chain, the *C* segment encodes the constant region of the chain, and the *J* segments encode a short set of nucleotides that join the *V* and *C* segments together. The number of *V*, *J*, and *C* segments differs among species. For the human kappa gene, there are 30 to 35 different functional *V* gene segments, 5 different *J* gene segments, and a single *C* gene segment, all of which are present in the germ-line DNA (Figure 22.23a). The *V* gene segments, which are about 400 bp in length, are all located on the same chromosome and are separated from one another by about 7000 bp. The *J* gene segments are each about 30 bp in length and together encompass about 1400 bp.



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22.23 Antibody diversity is produced by somatic recombination. Shown here is recombination among gene segments that encode the human kappa light chain.

Description
The illustration starts with a linear germline D N A. It has three regions labeled as follow: variable region segments showing four segments labeled V 1, V 2, V 3, V n; joining region segments showing five segments labeled J 1 through J 5; and a constant region segment showing one segment labeled C. A caption reads, “There are 30 to 35 different V gene segments, 5 J gene segments, and 1 C gene segment in germ-line D N A.” Then, a B-cell D N A is shown comprising of V 1, V 2, J 3, J 4, J 5, and C regions. The regions V 2, J 3, J 4, and J 5 are shown deriving from germline D N A. A caption reads, “In this example, V 2 is moved next to J 3 through somatic recombination, producing the D N A found in a mature B cell.” Then, transcription occurs and pre-m R N A is formed. It has the following regions: V 2, J 3, J 4, J 5, and C. A caption reads, “The V-J-C pre-m R N A is processed so that the mature m R N A contains sequences for only one V, J, and C gene segment.” A mature m R N A is formed then. It has V 2, J 3, and C. V 2 and J 3 are arising from the pre-m R N A. The mature R N A has poly-A tail at one end. A caption reads, “This m R N A is translated into a functional light chain.” Then, translation occurs and protein is formed having V, J, and C. The V in protein leads to variable region in light chain and C leads to constant region in light chain of an antibody.

Initially, an immature lymphocyte inherits all the *V* gene segments and all the *J* gene segments present in the germ line. In the maturation of the lymphocyte, **somatic recombination** within a single chromosome moves one of the *V* gene segments to a position next to one of the *J* gene segments. In **Figure 22.23b**, V_2 (the second of approximately 35 different *V* gene segments) undergoes somatic recombination, which places it next to J_3 (the third of 5 *J* gene segments); the intervening segments are lost.

After somatic recombination has taken place, the light-chain gene is transcribed into pre-mRNA that contains one *V* segment and several *J* segments, along with the *C* segment (**Figure 22.23c**). The resulting

pre-mRNA is processed (**Figure 22.23d**) to produce a mature mRNA that contains transcripts of only one *V*, one *J*, and one *C* segment; this mRNA is translated into a functional light chain (**Figure 22.23e**). In this way, each mature human B cell produces a unique type of kappa chain, and different B cells produce slightly different kappa chains, depending on the combination of *V* and *J* segments that are joined together.

The gene that encodes the lambda light chain is organized in a similar way but differs from the kappa-chain gene in the number of copies of the different segments. Somatic recombination takes place among the segments in the same way as that in the kappa gene, generating many possible combinations of lambda chains. The gene that encodes the immunoglobulin heavy chain is also arranged in *V*, *J*, and *C* segments, but this gene possesses *D* (for diversity) segments as well. Thus, many different types of light and heavy chains are possible.

Somatic recombination is brought about by RAG1 and RAG2 proteins, which generate double-strand breaks at specific nucleotide sequences, called recombination signal sequences, that flank the *V*, *D*, *J*, and *C* gene segments. DNA-repair proteins then process the ends of particular segments and join them together. Mutations in the genes that encode RAG1 and RAG2 proteins have been associated with some forms of severe combined immunodeficiency disease (SCID), a disease that severely limits immunity and affects development.

Other mechanisms in addition to somatic recombination add to antibody diversity. First, each type of light chain can potentially combine with each type of heavy chain to make a functional immunoglobulin molecule, increasing the amount of possible variation in antibodies. Second, the recombination process that joins *V*, *J*, *D*, and *C* gene segments in the developing B cell is imprecise, and a few random nucleotides are frequently lost or gained at the junctions of the recombining segments. This **junctional diversity** greatly enhances variation among antibodies. A third mechanism that adds to antibody diversity is **somatic hypermutation**, a process that leads to a high mutation rate in the antibody genes. This process is initiated when cytosine bases are deaminated and converted into uracil. The uracil bases are detected and replaced by DNA-repair mechanisms (see Section 18.5) that are error prone and often replace the original cytosine with a different base, leading to a mutation. These processes—somatic recombination, combining of heavy and light chains, junctional diversity, and somatic hypermutation—provide the possibility of producing at least 10^{11} different antibody specificities in humans. Through these processes, each lymphocyte comes to possess a unique set of genetic information (different from that in other lymphocytes) that encodes an antibody specific to a particular antigen. › **TRY PROBLEM 30**

CONCEPTS

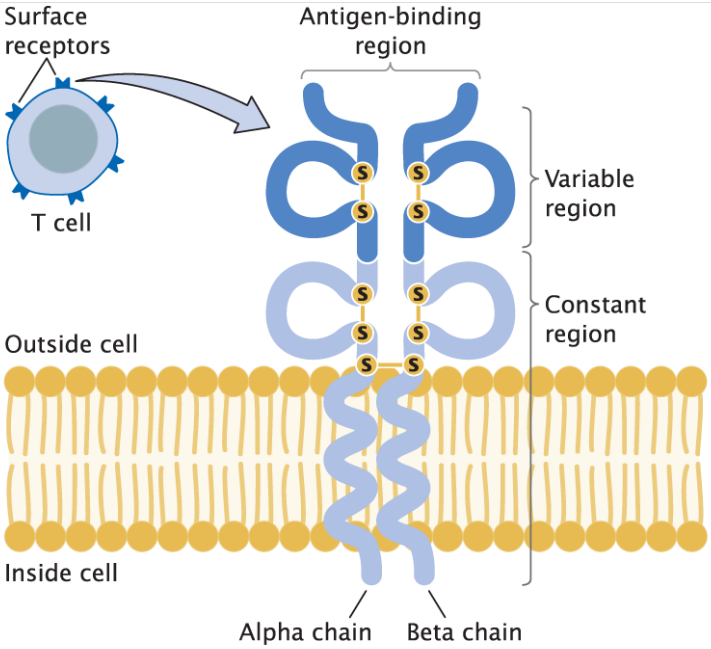
The genes encoding the chains that make up antibodies are organized in segments, and germ-line DNA contains multiple versions of each segment. The many possible combinations of *V*, *J*, and *D* segments permit an immense variety of different antibodies to be generated. This diversity is augmented by different combinations of light and heavy chains, the random addition and deletion of nucleotides at the junctions of the segments, and the high mutation rates in immunoglobulin genes.

✔ CONCEPT CHECK 7

How does somatic recombination differ from alternative splicing of RNA?

T-Cell-Receptor Diversity

Like a B cell, each mature T cell has genetically determined specificity for one type of antigen that is mediated through the cell's receptors. T-cell receptors are structurally similar to immunoglobulins (**Figure 22.24**) and are located on the cell surface. Most T-cell receptors are composed of one alpha and one beta polypeptide chain held together by disulfide bonds. One end of each chain is embedded in the cell membrane; the other end projects away from the cell and binds antigens. Like the immunoglobulin chains (see Figure 22.22), each chain of the T-cell receptor possesses a constant region and a variable region; the variable regions of the two chains provide the antigen-binding site.



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22.24 A T-cell receptor is composed of two polypeptide chains, each having a variable and a constant region. Most T-cell receptors are composed of alpha and beta polypeptide chains held together by disulfide bonds. One end of each chain traverses the cell membrane; the other end projects away from the cell and binds antigens.

Description

The alpha and beta chain are mirror images of each other. The extracellular portion of each polypeptide contains 2 hairpin-shaped loops. The alpha and beta chain are united by one disulfide bond on the very bottom of the extracellular domain, lying next to the lipid bilayer. Each of the hairpin loops are stabilized with a disulfide bond and the top of the structure looks like a pincher. The region of the protein going through the lipid bilayer up to the first hairpin loop is labeled the constant region. The top loop and pincher are labeled variable region.

The genes that encode the alpha and beta chains of the T-cell receptor are organized much like those that encode the heavy and light chains of immunoglobulins: each gene is made up of segments

that undergo somatic recombination before the gene is transcribed. For example, the human gene for the alpha chain initially consists of 44 to 46 *V* gene segments, 50 *J* gene segments, and a single *C* gene segment. These gene segments undergo somatic recombination similar to that in antibody genes; the process also requires RAG1 and RAG2 proteins. The organization of the gene for the beta chain is similar, except that it also contains *D* gene segments. Alpha and beta chains combine randomly, and there is junctional diversity, but there is no evidence for somatic hypermutation in T-cell-receptor genes.

THINK-PAIR-SHARE Question 7

CONCEPTS

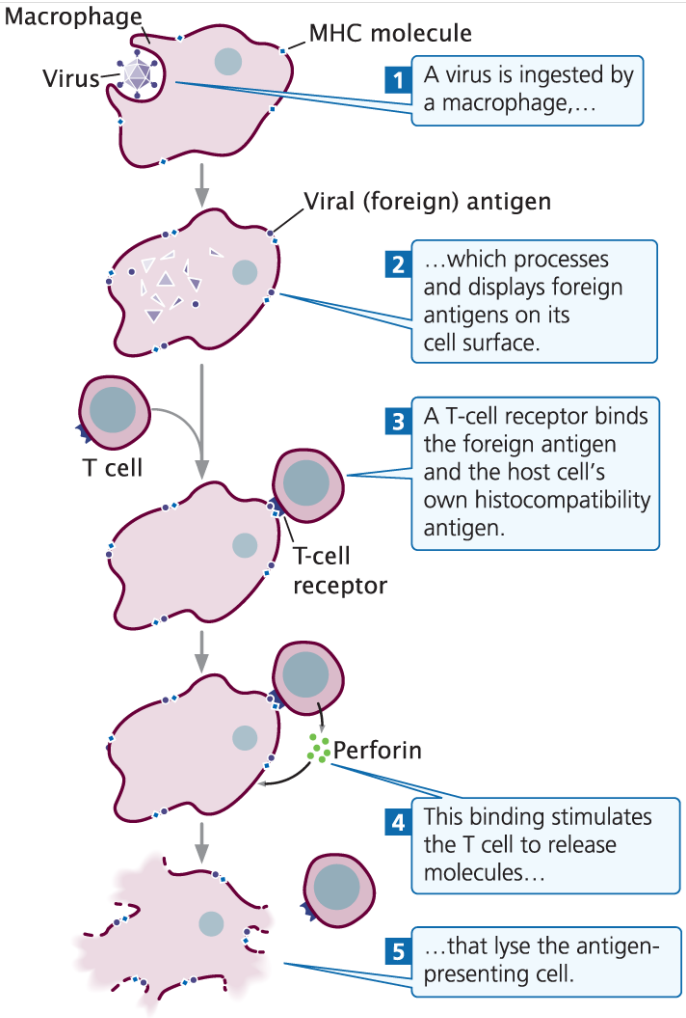
Like the genes that encode antibodies, the genes for the T-cell-receptor chains consist of segments that undergo somatic recombination, generating an enormous diversity of antigen-binding sites.

Major Histocompatibility Complex Genes

When tissues are transferred from one vertebrate species to another, or even from one member to another within a species, the

transplanted tissues are usually rejected by the host animal. The results of early studies demonstrated that this graft rejection is due to an immune response that takes place when antigens on the surface of cells of the grafted tissue are detected and attacked by T cells in the host animal. The antigens that elicit graft rejection are referred to as histocompatibility antigens, and they are encoded by a cluster of genes called the major histocompatibility complex (MHC).

T cells are activated only when the T-cell receptor simultaneously binds both its specific foreign antigen and one of the host cell's own histocompatibility antigens. The reason for this requirement is not clear; it may reserve T cells for action against pathogens that have invaded cells. When a foreign body, such as a virus, is ingested by a macrophage or another cell, partly digested pieces of the foreign body, containing antigens, are displayed on the cell's surface complexed with MHC molecules (**Figure 22.25**). A cell infected with a virus may also express viral antigens on its surface. Through their T-cell receptors, T cells bind to both the histocompatibility antigen and the foreign antigen and secrete substances that either destroy the antigen-containing cell, activate other B and T cells, or do both.



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22.25 T cells are activated by binding both to a foreign antigen and to a histocompatibility antigen on the surface of a self-cell.

Description

The flowchart starts with a virus being ingested by a macrophage, which is labeled M H C molecule. A caption reads, "A virus is ingested by a macrophage, which processes and displays foreign antigen on its cell surface." The flowchart then shows a virus inside the macrophage. T-cell is shown next, which attaches to receptor on macrophage. A caption reads, "A T-cell receptor binds the foreign antigen and the host cell's own histocompatibility (M H C) molecules." Perforins are secreted by the attached T-cell and the macrophage is lysed. A caption reads, "This binding stimulates the T cell to release molecules that lyse the antigen-presenting cell."

The MHC genes are among the most variable genes known: there are more than 100 different alleles for some MHC loci. Because each person possesses five or more MHC loci and because many alleles are possible at each locus, no two people (with the exception of identical twins) produce the same set of histocompatibility antigens. The variation in histocompatibility antigens provides each of us with a unique identity for our own cells, which allows our immune systems to distinguish self from nonself. This variation is also the cause of rejection in organ transplants.

CONCEPTS

The MHC genes encode proteins that provide identity to the cells of each individual. To bring about an immune response, a T-cell receptor must simultaneously bind both a histocompatibility (self) antigen and its specific foreign antigen.

Genes and Organ Transplants

For a person with a seriously impaired organ, a transplant operation may offer the only hope of survival. Successful transplantation requires more than the skills of a surgeon; it also requires a genetic match between the patient and the person donating the organ. The fate of transplanted tissue depends largely on the type of antigens present on the surface of its cells. Because foreign tissues are

usually rejected by the host, the successful transplantation of tissues between different people is very difficult. Tissue rejection can be partly inhibited by drugs that interfere with cellular immunity. Unfortunately, this treatment can create serious problems for transplant patients because they may have difficulty fighting off common pathogens and may thus die of infection. The only other option for controlling the immune reaction is to carefully match the donor and the recipient, maximizing their genetic similarities.

The tissue antigens that elicit the strongest immune reaction are the very ones used by the immune system to mark its own cells: those encoded by the major histocompatibility complex. In humans, the MHC spans a region of more than 3 million base pairs on chromosome 6 and has many alleles, providing different MHC antigens on the cells of each person and allowing the immune system to recognize foreign cells. The severity of an immune rejection of a transplanted organ depends on the number of MHC antigens on the cells of the transplanted tissue that fail to match those of the recipient. The ABO red-blood-cell antigens are also important because they elicit a strong immune reaction. The ideal donor is the patient's own identical twin, who will have exactly the same MHC and ABO antigens. Unfortunately, most patients don't have an identical twin. The next-best donor is a sibling with the same MHC and ABO antigens. If a sibling is not available, donors from the general population are considered. An attempt is made to match as many of the MHC antigens of the donor and recipient as

possible, and immunosuppressive drugs are used to control rejection due to the mismatches. The long-term success of transplants depends on the closeness of the match. Survival rates after kidney transplants (the most successful of the major organ transplants) increase from 63% with zero or one MHC match to 90% with four matches.

Scientists have now been successful in inducing adult cells to lose their specialized characteristics and return to an undifferentiated state (induced pluripotent stem cells; see Section 21.3), in which they are capable of developing into many different cell types. In the future, it may be possible to create pluripotent stem cells from a person's adult cells, then grow those cells into tissues or organs that could be transplanted back into the same person. Such cells would have the same MHC antigens as the original cell, avoiding the immune rejection that occurs with transplants between different people.

THINK-PAIR-SHARE Question 8

Chapter 22 Review

CHAPTER SUMMARY

Section 22.1 Learning Objective: Be able to provide evidence that genetic information is not lost during cell differentiation.

- Each multicellular organism begins as a single cell that has the potential to develop into any cell type. As development proceeds, cells become committed to particular fates. The results of cloning experiments demonstrated that this process arises from differential gene expression.

Section 22.2 Learning Objective: Be able to summarize the different groups of genes that lead to pattern formation in *Drosophila* and their general effects on development.

- In the early *Drosophila* embryo, pattern formation is brought about through a cascade of genetic control.
- The dorsal–ventral and anterior–posterior axes of the *Drosophila* embryo are established by egg-polarity genes, which are expressed in the female parent and produce mRNAs and proteins that are deposited in the egg cytoplasm. Initial differences in the distribution of these molecules regulate gene expression in various parts of the embryo. The dorsal–ventral axis is defined by a concentration gradient of the Dorsal

protein, and the anterior–posterior axis is defined by concentration gradients of the Bicoid and Nanos proteins.

- Three types of segmentation genes act sequentially to determine the number and organization of the embryonic segments in *Drosophila.* The gap genes establish large sections of the embryo, the pair-rule genes affect alternate segments, and the segment-polarity genes affect the organization of individual segments. Homeotic genes then define the identity of individual *Drosophila* segments.

Section 22.3 Learning Objective: Be able to describe the genetic control of flower development in angiosperms.

- Homeotic genes control the development of flower structure. Groups of genes interact to determine the identity of the four whorls found in a complete flower.

Section 22.4 Learning Objective: Be able to explain how programmed cell death is controlled through the regulation of apoptosis.

- Apoptosis, or programmed cell death, is a highly regulated process that depends on caspases—proteins that cleave other proteins. Apoptosis plays an important role in the development of many animals.

Section 22.5 Learning Objective: Be able to explain how the same genes in distantly related organisms can shape similar developmental pathways.

- Common genes are often seen affecting the same developmental pathways in distantly related organisms.
- Evolutionary changes often occur through changes in gene expression.

Section 22.6 Learning Objective: Be able to describe how the immune system is able to respond to a large number of potential antigens.

- The immune system is the primary defense network in vertebrates. In humoral immunity, B cells produce antibodies that bind foreign antigens; in cellular immunity, T cells attack cells carrying foreign antigens.
- Each B and T cell is capable of binding only one type of foreign antigen. When a lymphocyte binds to an antigen, the lymphocyte divides and gives rise to a clone of cells, each specific for that same antigen—the primary immune response. A few memory cells remain in circulation for long periods of time and, on exposure to that same antigen, can proliferate rapidly and generate a secondary immune response.
- Immunoglobulins (antibodies) are encoded by genes that consist of several types of gene segments; germ-line DNA contains multiple copies of these gene segments, which differ slightly in sequence. Somatic recombination randomly brings together one version of each segment to produce a single complete gene, allowing many combinations. Diversity is further increased by the random addition and deletion of nucleotides at the junctions of the segments and by a high mutation rate.

- The germ-line genes for T-cell receptors consist of segments with multiple varying copies. Somatic recombination generates many different types of T-cell receptors in different cells. Junctional diversity also adds to T-cell-receptor variation.
- The major histocompatibility complex encodes a number of antigens. The MHC antigens allow the immune system to distinguish self from nonself. Each locus for the MHC contains many alleles.

WORKED PROBLEMS

Problem 1

If a fertilized *Drosophila* egg is punctured at the anterior end and a small amount of cytoplasm is allowed to leak out, what will be the most likely effect on the development of the fly embryo?

» Solution Strategy

What information is required in your answer to the problem?

The likely effect on development of removing cytoplasm from the anterior end of a fertilized fly egg.

What information is provided to solve the problem?

Cytoplasm is removed from the anterior end.

For help with this problem, review:

Egg-Polarity Genes in Section 22.2.

» Solution Steps

The egg-polarity genes determine the major axes of development in the *Drosophila* embryo. One of these genes is *bicoid*, which is transcribed in the maternal ovary. After fertilization, Bicoid protein forms a concentration gradient along the anterior–posterior axis of the embryo. The high concentration of Bicoid protein at the anterior end induces the development of anterior structures, such as the head of the fruit fly. If the anterior end of the egg is punctured, cytoplasm containing high concentrations of *bicoid* mRNA and Bicoid protein will leak out, reducing the concentration of Bicoid protein at the anterior end. The result will be that the embryo fails to develop head and thoracic structures at the anterior end.

> **Recall:** The *bicoid* gene is an egg-polarity gene that helps determine the anterior–posterior axis of the developing embryo.

Problem 2

The immunoglobulin molecules of a particular mammalian species have kappa and lambda light chains and heavy chains. The kappa gene consists of 250 *V* and 8 *J* segments. The lambda gene contains 200 *V* and 4 *J* segments. The gene for the heavy chain consists of 300 *V*, 8 *J*, and 4 *D* segments. If just somatic recombination and random combinations of light and heavy chains are taken into consideration, how many different types of antibodies can be produced by this species?

» Solution Strategy

What information is required in your answer to the problem?

The number of different types of antibodies that can be produced if somatic recombination and random combinations of light and heavy chains are considered.

What information is provided to solve the problem?

- The kappa gene has 250 *V* and 8 *J* segments.
- The lambda gene has 200 *V* and 4 *J* segments.
- The heavy chain has 300 *V*, 8 *J*, and 4 *D* segments.

For help with this problem, review:

The Generation of Antibody Diversity in Section 22.6.

» Solution Steps

For the kappa light chain, there are 250 × 8 = 2000 combinations; for the lambda light chain, there are 200 × 4 = 800 combinations; so a total of 2800 different types of light chains are possible. For the heavy chains, there are 300 × 8 × 4 = 9600 possible types. Any of the 2800 light chains can combine with any of the 9600 heavy chains, so there are 2800 × 9600 = 26,880,000 different types of antibodies possible from somatic recombination and random chain combination alone. Junctional diversity and somatic hypermutation would greatly increase this diversity.

Hint: The number of each type of light chain consists of the number of *V* segments times the number of *J* segments.

Hint: To determine the number of different types of antibodies, multiply the number of possible light chains by the number of possible heavy chains.

IMPORTANT TERMS

totipotency
determination
egg-polarity gene
morphogen
segmentation gene

gap gene
pair-rule gene
segment-polarity gene
homeotic gene
homeobox
Antennapedia complex
bithorax complex
homeotic complex (HOM-C)
Hox gene
apoptosis
caspase
antigen
autoimmune disease
humoral immunity
B cell
antibody
T cell
cellular immunity
T-cell receptor
major histocompatibility complex (MHC) antigen
theory of clonal selection
primary immune response
memory cell
secondary immune response
somatic recombination
junctional diversity
somatic hypermutation

ANSWERS TO CONCEPT CHECKS

1. No, it does *not* prove that genetic material is not lost during development, because differentiation has not yet taken place in an early embryo. The early embryo would still be likely to contain all its genes, and its nucleus could therefore give rise to a complete animal. The use of specialized cells, such as a cell from an udder, does prove that genes are not lost during development, because if they were lost, there would be no cloned animal.
2. c
3. b
4. d
5. d
6. In cell death from necrosis, the cell swells and bursts, causing an inflammatory response. In cell death through apoptosis, the cell's DNA is degraded, its nucleus and cytoplasm shrink, and the cell is phagocytized, without leakage of cellular contents.
7. Somatic recombination takes place through the rearrangement of DNA segments, so each lymphocyte has a different sequence of nucleotides in its DNA. Alternative splicing (see Section 14.2) takes place through the rearrangement of RNA sequences in pre-mRNA; there is no change in the DNA that encodes the pre-mRNA. The generation of antibody diversity requires both somatic recombination of DNA sequences and alternative splicing of pre-mRNA sequences.

Chapter 22 Assessment

COMPREHENSION QUESTIONS

Section 22.1

1. What experiments suggested that genes are not lost or permanently altered in development?

Section 22.2

2. Briefly explain how the Dorsal protein is redistributed in the formation of the *Drosophila* embryo and how this redistribution helps to establish the dorsal–ventral axis of the fruit fly.

3. Briefly describe how the *bicoid* and *nanos* genes help to determine the anterior–posterior axis of the fruit fly.

4. List the three major classes of segmentation genes, and outline the function of each.

5. What role do homeotic genes play in the development of fruit flies?

Section 22.3

6. How do class A, class B, and class C genes in plants work together to determine the structures of the flower?

Section 22.4

7. What is apoptosis, and how is it regulated?

Section 22.6

8. Explain how each of the following processes contributes to antibody diversity.
   a. Somatic recombination
   b. Junctional diversity
   c. Hypermutation

9. What is the function of the MHC antigens? Why are the genes that encode these antigens so variable?

APPLICATION QUESTIONS AND PROBLEMS

Section 22.1

10. CONNECT If telomeres are normally shortened after each round of replication in somatic cells (see Chapter 12), what prediction would you make about the length of telomeres in Dolly, the first cloned sheep?

Section 22.2

11. A drug causes the degradation of Cactus protein. What would be the effect of administering this drug to developing *Drosophila* embryos?

12. What would be the effect of deleting the *toll* gene in *Drosophila* embryos?

13. CONNECT Why is it that mutations in *bicoid* and *nanos* exhibit genetic maternal effect in *Drosophila* (a mutation in the maternal parent produces a phenotype that shows up in the offspring; see Section 5.3), but mutations in *runt* and *gooseberry* do not? (Hint: See **Tables 22.3** and **22.4**.)

14. CONNECT *Give examples of genes that affect development in fruit flies by regulating gene expression at the level of (a) transcription (see Chapter 13) and (b) translation (see Chapter 15).

15. Using **Figure 22.6**, indicate the stage at which segmentation genes, homeotic genes, and egg-polarity genes would have an effect on development.

16. What would be the most likely effect on development of puncturing the posterior end of a *Drosophila* egg, allowing a

small amount of cytoplasm to leak out, and then injecting that cytoplasm into the anterior end of another egg?

17. DATA ANALYSIS Christiane Nüsslein-Volhard and her colleagues carried out several experiments in an attempt to understand what determines the anterior and posterior ends of a *Drosophila* larva (reviewed in C. Nüsslein-Volhard, H. G. Frohnhofer, and R. Lehmann. 1987. *Science* 238:1675–1681). They isolated fruit flies with mutations in the *bicoid* gene (bcd^-). These flies produced embryos that lacked a head and thorax. When they transplanted cytoplasm from the anterior end of an egg from a wild-type female into the anterior end of an egg from a mutant *bicoid* female, normal head and thorax development took place in the embryo. However, transplanting cytoplasm from the posterior end of an egg from a wild-type female into the anterior end of an egg from a *bicoid* female had no effect. Explain these results in regard to what you know about proteins that control the determination of the anterior–posterior axis.

18. What would be the most likely result of injecting *bicoid* mRNA into the posterior end of a *Drosophila* embryo and inhibiting the translation of *nanos* mRNA?

19. CONNECT What would be the most likely effect of inhibiting the translation of *hunchback* mRNA throughout a *Drosophila* embryo by using RNAi (see Chapter 19)?

20. *Molecular geneticists have performed experiments in which they altered the number of copies of the *bicoid* gene in flies, affecting the amount of Bicoid protein produced.
    a. What would be the effect on development of an increased number of copies of the *bicoid* gene?
    b. What would be the effect of a decreased number of copies of *bicoid*? Justify your answers.

21. What would be the most likely effect on fruit-fly development of a deletion in the *nanos* gene?

22. *Give an example of a gene found in each of the categories of genes (egg-polarity, gap, pair-rule, and so forth) listed in **Figure 22.13**.

23. CONNECT In Chapter 1, we considered preformationism, the early idea about heredity that suggested that inside the egg or sperm is a tiny adult called a homunculus, with all the features of an adult human in miniature. According to this idea, the homunculus simply enlarges during development. What types of evidence presented in this chapter prove that preformationism is false?

Section 22.3

24. Explain how (a) the absence of class B gene expression produces the flower structures seen in class B mutants (see **Figure 22.15c**) and (b) the absence of class C gene expression

produces the structures seen in class C mutants (see **Figure 22.15d**).

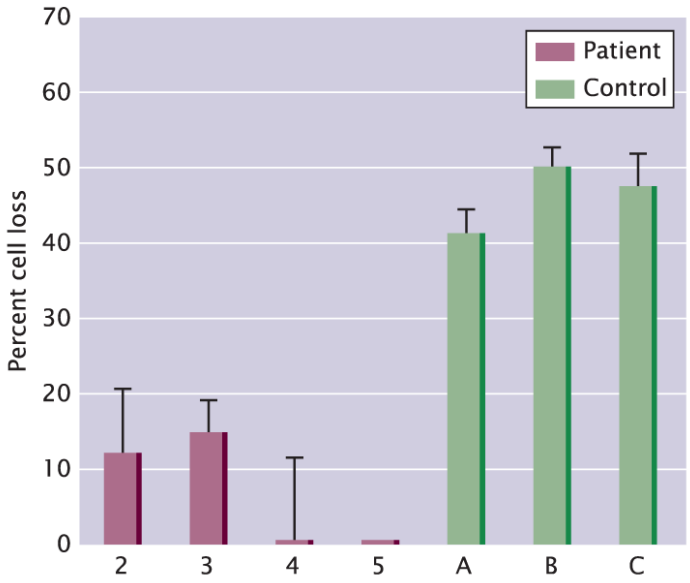
25. *What would you expect a flower to look like in a plant that lacked both class A and class B genes? In a plant that lacked both class B and class C genes?

26. What will be the flower structure of a plant in which expression of the following genes is inhibited in the specified whorls?
    a. Expression of class B genes is inhibited in the second whorl but not in the third whorl.
    b. Expression of class C genes is inhibited in the third whorl but not in the fourth whorl.
    c. Expression of class A genes is inhibited in the first whorl but not in the second whorl.
    d. Expression of class A genes is inhibited in the second whorl but not in the first whorl.

Section 22.4

27. DATA ANALYSIS Autoimmune lymphoproliferative syndrome (ALPS) is an inherited disease in which the body does not properly regulate the production of immune cells. It is characterized by the generation of abnormally large numbers of certain lymphocytes. The accumulation of these cells leads to enlargement of lymph nodes, liver, and spleen. Individuals with ALPS often develop problems of autoimmunity, in which their immune system attacks their own tissues. To investigate the cause of ALPS, researchers conducted an experiment in which they induced apoptosis and measured cell loss in blood

lymphocytes of four patients with ALPS and three control individuals who did not have the disease (G. H. Fisher et al. 1995. *Cell* 81:935–946). The results of this experiment are shown on the next page. What conclusions can you draw from these results?



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Data from G. H. Fisher et al. 1995. *Cell* 81:935–946.

Description

The horizontal-axis represents four patients numbered “2” through “5” and three control individuals labeled “A” through “C.” The vertical-axis represents percent cell loss ranging from 0 to 70 with increments of 10. Approximate data from the graph are as follows:

Patient 2: 12 (bar extending to 21)

Patient 3: 15 (bar extending to 19)

Patient 4: 2 (bar extending to 12)

Patient 5: 2

Control A: 42 (bar extending to 45)

Control B: 50 (bar extending to 53)

Control C: 47 (bar extending to 52)

Section 22.5

28. DATA ANALYSIS *William Jeffrey and his colleagues crossed surface-dwelling Mexican tetras that had fully developed eyes with cave-dwelling blind Mexican tetras. The progeny from this cross had uniformly small eyes compared with those of surface fish (Y. Yamamoto, D. W. Stock, and W. R. Jeffrey. 2004. *Nature* 431:844–847). What prediction can you make about the expression of *shh* in these progeny at the embryonic stage relative to its expression in embryonic surface fish?

29. CONNECT *The zebrafish is a genetic model organism. Geneticists have identified several mutations in zebrafish, called *TET* mutations, that are defective in an enzyme that normally demethylates DNA. DNA methylation is an epigenetic modification that represses gene expression (see Chapter 21). Based on the role of DNA methylation in eye development of blind Mexican tetras, what, if any, effect do you think these mutations might have on eye size in zebrafish?

Section 22.6

30. *In a particular species, the gene for the kappa light chain has 200 *V* segments and 4 *J* segments. In the gene for the lambda light chain, this species has 300 *V* segments and 6 *J* segments. If only the antibody diversity arising from somatic recombination is taken into consideration, how many different types of light chains are possible?

31. Based on the information provided in **Figure 22.21**, what would be the likely effect of a mutation that prevented the formation of memory cells?

CHALLENGE QUESTIONS

Section 22.2

32. As we have learned in this chapter, the Nanos protein inhibits the translation of *hunchback* mRNA, lowering the concentration of Hunchback protein at the posterior end of a fruit-fly embryo and stimulating the differentiation of posterior characteristics. The results of experiments have demonstrated that the action of Nanos on *hunchback* mRNA depends on the presence of an 11-base sequence that is located in the 3′ untranslated region (3′ UTR) of *hunchback* mRNA. This sequence has been termed the Nanos response element (NRE). There are two copies of NRE in the 3′ UTR of *hunchback* mRNA. If a copy of NRE is added to the 3′ UTR of another mRNA produced by a different gene, that mRNA is repressed by Nanos. The repression is greater if several

NREs are added. On the basis of these observations, propose a mechanism for how Nanos inhibits Hunchback translation.

33. Compare and contrast the number and organization of *Hox* genes in *Drosophila* with those in mammals. (Hint: See **Figure 22.12**.) What might account for the similarities in types and arrangement of *Hox* genes of *Drosophila* and mammals?

Section 22.6

34.

Ataxia-telangiectasis (ATM) is a rare genetic neurodegenerative disease. About 20% of people with ATM develop acute lymphocytic leukemia or lymphoma, cancers of the immune-system cells. Cells in many of these cancers exhibit chromosome rearrangements, with chromosome breaks occurring at antibody and T-cell-receptor genes (A. L. Bredemeyer et al. 2006. *Nature* 442:466–470). Many people with ATM also have a weakened immune system, which makes them susceptible to respiratory infections. Research has shown that the locus that causes ATM has a role in the repair of double-strand breaks. Explain why people who have a genetic defect in the repair of double-strand breaks might have a high incidence of chromosome rearrangements in their immune-system cells and why their immune systems might be weakened.

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 22.1

1. Although a number of different animals have been successfully cloned, the process of creating cloned animals is very inefficient. Typically, only about 0.1% to 3% of attempts result in a live-born animal. What might be some of the reasons for this low rate of success?

2. Research has now demonstrated that techniques that have been developed for cloning animals could be used to clone human embryos, although no living human clones have been produced. There is widespread consensus that cloning a live human would be unethical. However, some people have suggested creating cloned embryos from which stem or pluripotent cells, which have the ability to develop into any tissue or organ, could be extracted. The stem and pluripotent cells might then be used to create genetically identical tissues and organs that could be transplanted back into the donor to treat failed organs and diseases. What might be some reasons for or against this type of therapeutic cloning?

Section 22.2

3. Propose one or more explanations for why *Hox* genes exhibit a relation between their order on the chromosome and the timing of their expression, with *Hox* genes at one end of the complex expressed early and those at the other end expressed later.

Section 22.3

4. Flowers are complex and beautiful structures that are highly variable among plant species, differing in color and size and in the number and arrangement of their basic parts: the sepals, petals, stamens, and carpels. Why do plants go to the trouble of producing these complex structures, and why do flowers vary so much among different plant species?

Section 22.4

5. Apoptosis in the frog *Xenopus laevis* has been the subject of research. Can you think of some reasons why apoptosis has been studied in frogs?

Section 22.6

6. Childhood vaccinations are required by law in all U.S. states and in many countries to prevent the outbreak and spread of infectious diseases. Some people object to required vaccinations and feel that whether to vaccinate or not should be a personal choice. What are some arguments for and against requiring everyone to be vaccinated?

7. Severe combined immunodeficiency disease is a life-threatening genetic disease in which children are born with defective B-cell and T-cell function. These children are susceptible to severe infections, such as pneumonia, meningitis, or blood-borne infections. They can also be infected and sickened by live viruses that are present in some vaccines. Children with some forms of severe combined immunodeficiency disease have

mutations in their *RAG1* or *RAG2* genes. Provide a possible explanation for how mutations in these genes might lead to the symptoms of this disorder.

8. In the novel *Chromosome 6*, by Robin Cook, a biotechnology company genetically engineers individual bonobos (a type of chimpanzee) to serve as future organ donors for clients. The genes of the bonobos are altered so that no tissue rejection takes place when their organs are transplanted into a client. What genes would need to be altered for this scenario to work? Explain your answer.

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Draw a concept map that outlines the genetic mechanism by which stickleback fish have lost their pelvic spines during their evolutionary transition from marine to freshwater environments.

Section 22.2

2. Create a concept map that summarizes the genetic control of pattern formation in *Drosophila*. Include the following terms in your concept map: *Drosophila* embryo, main body axes, dorsal–

ventral axis, anterior–posterior axis, number and polarity of segments, identity of segments, *dorsal*, *bicoid*, egg-polarity genes, segmentation genes, homeotic genes, pair-rule genes, segment-polarity genes, gap genes, *Antennapedia* complex, *bithorax* complex.

Section 22.3

3. Draw a concept map that includes the different anatomical parts of a flower and summarizes the classes of genes that control the development of these flower parts.

Section 22.4

4. Create a concept map that explains how apoptosis controls cell death in animals. Include at least the following terms in your concept map: cell death, apoptosis, necrosis, caspases, procaspase, protein cleavage, caspase genes, DNase.

Section 22.6

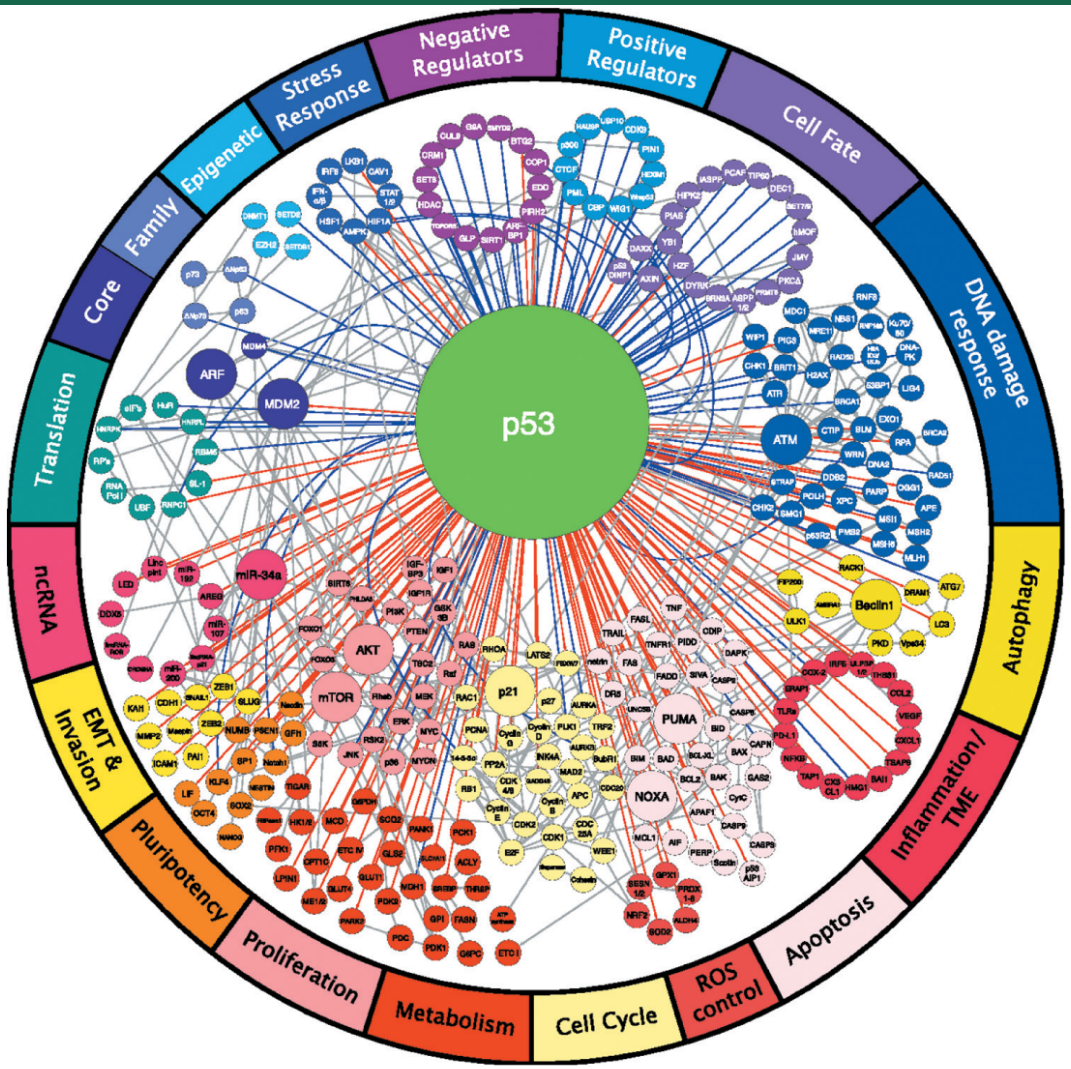
5. Draw a concept map that summarizes the organization of the immune system, including the following terms: immune system, humoral immunity, cellular immunity, lymphocytes, B cells, antigen, antibody, T cells, bone marrow, thymus, T-cell receptors, MHC antigens, primary immune response, secondary immune response, memory cells.

6. Create a concept map that explains how antibody diversity is generated by the immune system. Include the following terms in your map: immunoglobulin, light chains, heavy chains, kappa chains, lambda chains, *V* segments, *J* segments, *C* segments, immature lymphocyte, somatic recombination, variation in mature B cells, RAG1, RAG2, junctional diversity, somatic hypermutation.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 23

Cancer Genetics



Republished with permission of Elsevier, from Kastenhuber, ER, Lowe, SW, "Putting p53 in Context," Cell, 2017, September 7; 170 (6): 1062–1078, Figure 1. Permission conveyed through Copyright Clearance Center, Inc.

The p53 protein, called the "guardian of the genome," has a number of functions in the cell. p53, at the center in green, affects many biological processes, shown in the outer circles. The small circles are genes with which p53 interacts; each line represents an interaction.

Description

The illustration shows p 53 protein as a small green circle at the center surrounded by two outer concentric circles. The two outer circles are divided into 19 segments. Each segment has a function written inside as follows: Cell fate, D N A damage response, autophagy, inflammation/T M E, apoptosis, R O S control, cell cycle, metabolism, proliferation, pluripotency, E M T and invasion, n c R N A, translation, core, family, epigenetic, stress response, negative regulators, and positive regulators. For each of these functions, there are lines arising from the green p 53 protein interacting with several genes as follows:

For cell fate, the genes are as follows: P C A F, D E C 1, T I P 60, S E T 779, h M O F, J M Y, p K C A, P R M T 8, A B P P 1 over 2, B F L N 3 A, D Y R K, H Z F, Y B 1, P 1 A 6, H P K 2, I A S P P, A X N, D A X X, p 53 DIN P1.

For D N A damage response, the genes are as follows: M D C 1, N B S 1, M R E 11, R N F 8, Ku 70/80, D N A P K, LIG-4, RAD-50, EXO 1, B R C A 2, RAD-51, A P E, M S 11, M S H 8, M S H 2, M L H 1, P M S 2, p 53 R z, P O L H, A T M, C H K 2, S M G 1, D D B 2, H 2 A X, A T R, C H K 1, W I P 1, BRIT-1, PIG-3, M D C-1, and some other genes.

For autophagy, the genes are as follows: F I P 200, U L K 1, R A C K 1, D R A M 1, Beclin 1, P K D, V p e 34, L C 3, A T G 7, and one other gene.

For inflammation/T M E, the genes are as follows: T L R s, E R A P 1, C C E X 2, I R F 5, U L P B P 1 over 2, T H B S 1, C C L 2, V E G F, C X C L 1, T SAP 8, BAL, H M G 1, C X S C L 1, TAP 1, N F K B, and P D L 1.

For apoptosis, the genes are as follows: T N F, F A S L, TRAIL, P I D D, C D I P, T N F R 1, F A 8, D R s, F A D D, PUMA, D A P K, C A S P R, BID, BAX, CAP N, GAS 2, BAK, BAD, BIM, B C L-X L, A P A F 1, N O X A, M C L 1, A I F, P E R P, p 58 A J P 1, scotin, and other genes.

For R O S control, the genes are as follows: B E S N 1 over 2, G P X 1, PROX 1 to 8, A L D H 4, B O D 2, and N R F 2.

For cell cycle, the genes are as follows: p 21, R H O A, R A C 1, P C N A, LAT S 2, p 27, AURKA, cyclin B, cyclin D, P L K 1, T R F 2, C D C 20, A P C, N K 4 A, MAD 2, P P 2 A, R B 1, cyclin E, C D K 428, C D C 25 A, W E E 1, B 2 F, and other genes.

For metabolism, the genes are as follows: TIGAR, H K 1 over 2, P F K 1, L P I N 1, C P T 1 C, M C D, G L UT 1, G L U T 4, M E 1 over 2, P A R K 2, E T C M, S C O 2, G L S 2, P A N K 1, P D K 2, P D C, G P 1, A C L Y, P C K 1, E T C 1, G 6 P C, and other genes.

For proliferation, the genes are as follows: I G F 1, I G F B P 3, I G F 1 R, P 1 3 K, P T E N, B I R T 8, G 8 K 3 B, RAS, A K T, m T O R, M E K, M Y C, p 38, J N K, E R K, and other genes.

For pluripotency, the genes are as follows: G F M, NUMB, S P 1, P S E N 1, SOX 2, K L F 4, O C T 4, L I F, NANO G, and other genes.

For E M T and invasion, the genes are as follows: ZEB 1, SLUG, SNAIL 1, ZEB 2, C D H 1, I C A M 1, K A H, and other genes.

For n c R N A, the genes are as follows: m I R-34 a, A R E G, m I R-107, m I R-200, D D X 8, L E D, Linc pint, m I R-192. And other genes.

For translation, the genes are as follows: H U R, R B M S, H N R P L, S L-1, R N P C 1, U B F, R N A Pol 1, and other genes.

For core, the genes are as follows: A R F, M D M 2, and M D M 4.

For family, the genes are as follows: p 73, p 53, and two other genes.

For epigenetic, the genes are as follows: 9 E T D 2, E Z H 2, 8 E T D 8, and D N M T 1.

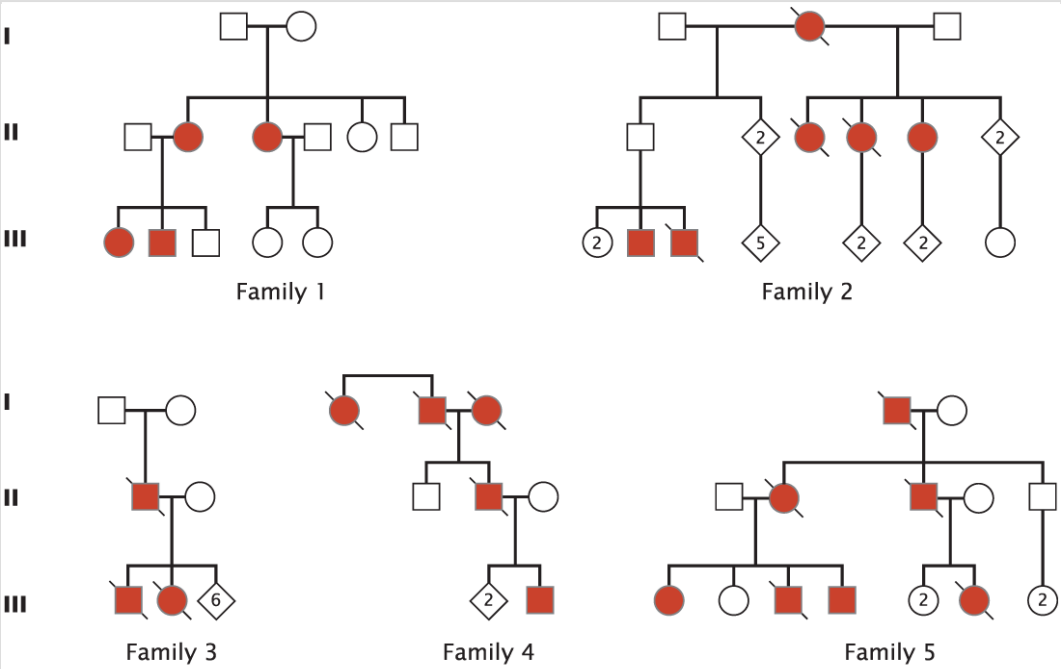
For stress response, the genes are as follows: L K B 1, C A V 1, S T A T 1 over 2, H I F 1 A, A M P K, H 9 F 1, I F N-alpha/beta, and I R F-8.

For negative regulators, the genes are as follows: B T G 2, COP 1, E D O, P I R H 2, A R F-B P 1, G L P, H D A C, SET 8, C R M 1, C U L B, and other genes.

For positive regulators, the genes are as follows: C D K 9, PIN 1, W I G 1, C B P, P M L, C T C F, U B P 10, and other genes.

Four Families and the Guardian of the Genome

In 1969, two American physicians, Frederick Pei Li and Joseph F. Fraumeni, were examining medical records of 648 children who had rhabdomyosarcoma, a rare type of cancer that occurs in the soft tissues, mostly muscles. Li and Fraumeni discovered something remarkable—some of the individuals in their database were siblings and cousins. Upon closer examination, they found four families in which a number of the family members exhibited striking histories of diverse types of cancer, including soft tissue sarcomas, breast cancer, brain tumors, and bone cancer. Li and Fraumeni recognized that these families exhibited a predisposition, not to a particular type of cancer but rather to cancer in general. Further research showed that their predisposition was inherited as a simple autosomal dominant trait (Figure 23.1). Li and Fraumeni described this condition as a new cancer syndrome, which later became known as Li-Fraumeni syndrome.



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23.1 Pedigrees of families with Li-Fraumeni syndrome. Cancer in these families is inherited as an autosomal dominant trait. [Data from Malkin et al. 1990. Germline *p53* mutations in a family syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 250:1233–1238.]

Description

The pedigree chart for family 1 is as follows: The male and female in the first generation are both unaffected. They produce three daughters and one son in generation 2. Two of the three daughters are affected. The two affected daughters mate with unaffected males. One of them produces a daughter and two sons; of which only one son is unaffected. The other produces two unaffected daughters.

The pedigree chart for family 2 is as follows: Two males mate with a deceased affected female in the first generation. The first mating produces one son and 2 unknown sex children in generation 2. Both are unaffected. The son produces 2 unaffected daughters and two affected sons, one of which is deceased. The two children of unknown sex produce 5 unaffected children of unknown sex. The second mating produces three affected daughters and 2 unaffected unknown sex children in generation 2. Two daughters are deceased. One deceased and another daughter produce 2 unaffected children of unknown sex in generation 3.

The pedigree chart for family 3 is as follows: The male and female in the first generation are both unaffected. They produce one affected deceased son in generation 2. This son mates with an unaffacted female and produces one affected deceased son, one affected deceased daughter, and 6 unaffected children of unknown sex in generation 3.

The pedigree chart for family 4 is as follows: The male and female in the first generation are both affected and deceased. They produce one affected deceased son and one unaffected son in generation 2. The affected son mates with an unaffacted female and produces one affected son and 2 unaffected children of unknown sex in generation 3.

The pedigree chart for family 5 is as follows: The male in the first generation is affected and deceased, while the female is unaffected. They produce one unaffected son, one affected deceased son, and one affected and deceased daughter in generation 2. The unaffected son produces 2 unaffected daughters. The affected son mates with unaffected female and produces 2 unaffected daughters and one affected and deceased daughter.

The affected daughter mates with unaffected male and produces 2 daughters (one affected and one unaffected) and 2 affected sons, one of which is deceased.

The families identified by Li and Fraumeni were exceptional. Most cancers do not develop until later in life, and most are not inherited. The study of Li-Fraumeni families, however, suggested that cancer could be inherited, and pointed the way toward our current understanding that cancer is, at its core, a genetic disease. As we will see, most cancers arise through the accumulation of several somatic mutations, mutations that are not passed on. However, sometimes people inherit a germ-line mutation in one of the genes that contributes to cancer progression. These individuals are predisposed to cancer because fewer additional somatic mutations are required for the cancer to develop.

Subsequent research on Li-Fraumeni families showed that many of these individuals inherited a germ-line mutation in their *TP53* gene, a gene that encodes a vital protein called p53. The p53 protein has been called the "guardian of the genome" because, through its multiple functions, p53 maintains the integrity of the genome and prevents cells from becoming cancerous. *TP53* is a tumor-suppressor gene, producing a protein that ensures that cell division only takes place under appropriate conditions. When the *TP53* gene is mutated, cell division is unregulated and abnormal, and tumors develop. Males with Li-Fraumeni syndrome have a 75% lifetime risk of cancer, and females have a lifetime risk of almost 100%. For these

people, the probability of developing cancer is 85 times greater than for those in the general population.

Mutations in *TP53* are not just associated with cancer in Li-Fraumeni families. Further research has shown that *TP53* plays an important role in spontaneous cancers as well. Approximately 50% of *all* tumors have mutations in *TP53*, and *TP53* mutations are associated with many different types of cancers. p53 is a DNA-binding protein that functions as a transcription factor, regulating transcription, and most of the mutations in *TP53* that are associated with cancer occur in the DNA-binding domain of p53.

The p53 protein has a number of different functions in a normal cell. It is critical for proper functioning of the G1/S checkpoint in the cell cycle. If DNA damage is present, p53 is activated and prevents the cell from moving into S phase, where DNA replication would normally take place. This arrest in the cell cycle allows time for DNA-repair mechanisms to correct the damage and prevents the propagation of mutations that might lead to cancer. When p53 is defective, this vital checkpoint is bypassed and damaged DNA gets perpetuated, leading to somatic mutations that contribute to the cancer process.

In ways that are not fully understood, p53 also helps to stabilize the genome. Cells with mutations in *TP53* are often aneuploid, containing too many or too few chromosomes. Thus, p53 helps maintain the integrity of the genome, probably by regulating

processes that normally lead to proper chromosome duplication and segregation. In yet another function, p53 affects apoptosis. When cells sustain DNA damage or develop abnormal chromosomes, apoptosis leads to cell death, eliminating these aberrant cells before they can multiply and become cancerous. However, when *TP53* is mutated, apoptosis does not occur normally, and aberrant cells continue to propagate. p53 also suppresses the movement of retrotransposons, which otherwise can insert into genes and cause mutations that may lead to cancer. Recent research demonstrates that p53 plays a role in a number of other critical functions and cellular pathways, such as metabolism and development. The bottom line is that p53 is critical to proper functioning of the cell; when *TP53* is mutated, things go wrong and cells become cancerous.

In spite of all the research that has focused on p53 and its role in cancer, there is, unfortunately, no cure for those with Li-Fraumeni syndrome, although their cancers can be treated. Even with the tremendous amount of research that has been devoted to understanding p53, physicians still have difficulty predicting when cancer will occur or what types of cancer may arise. Research into p53 has, however, greatly advanced our understanding of cancer in general.

THINK-PAIR-SHARE

- How might the *TP53* gene help to maintain genome stability? What are some specific processes or mechanisms that p53 might influence that would normally prevent the development of aneuploid cells?

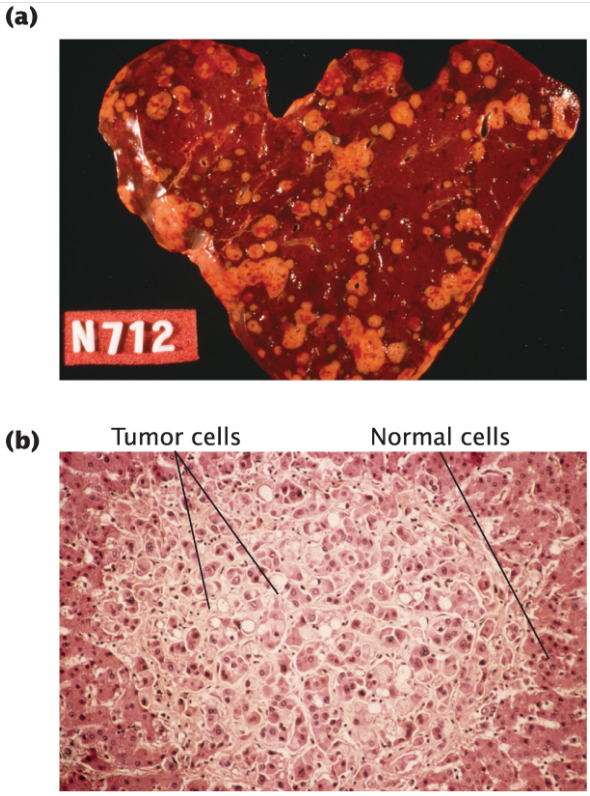
- Is it correct to say that the *TP53* gene causes cancer? Everyone has a *TP53* gene, but not everyone gets cancer. What might be a more accurate way to talk about the link between the *TP53* gene and cancer?

The discovery of p53's link to cancer illustrates the power of modern molecular genetics to unravel the biological nature of cancer. In this chapter, we examine the genetic nature of cancer, a disease that is fundamentally genetic but is not often inherited. We begin by considering the multiple genetic alterations required to transform a normal cell into a cancerous one. We then consider some of the types of genes that contribute to cancer, including oncogenes and tumor-suppressor genes, genes that control the cell cycle, genes encoding DNA-repair systems and telomerase, and genes that contribute to the spread of cancer. Next, we take a look at epigenetic changes associated with cancer and, as an example, we examine how specific genes contribute to the progression of colon cancer. Finally, we discuss chromosome mutations associated with cancer and the role of viruses in some cancers.

23.1 Cancer Is a Group of Diseases Characterized by Cell Proliferation

About one of every five people in the United States will die from cancer, and cancer treatments cost billions of dollars per year. Cancer is not a single disease; rather, it is a heterogeneous group of disorders characterized by the presence of cells that do not respond to the normal controls on division. Cancer cells divide rapidly and continuously, creating tumors that crowd out normal cells and eventually rob healthy tissues of nutrients (Figure 23.2). The cells of an advanced tumor can separate from the tumor and travel to distant sites in the body, where they may take up residence and develop into new tumors. The most common cancers in the United States are those of the prostate gland, breast, lung, colon and rectum, and blood (Table 23.1).

THINK-PAIR-SHARE Question 1



CNRI/Science Source.

23.2 Abnormal proliferation of cancer cells produces a tumor that crowds out normal cells. (a) Metastatic breast cancer masses (white protrusions) growing in a human liver. (b) A light micrograph of a liver section with tumors. The cancer cells are the light, pale-stained cells; the darker cells are healthy liver cells.

Description
Photo “a” shows a human liver with white spots. Text below reads, N 7 12. Photo “b” shows a micrograph in which tumor cells and normal cells are labeled. Normal cells are smaller than tumor cells.

TABLE 23.1 Estimated incidences of various cancers and cancer mortality in the United States in 2019

Type of Cancer	New Cases per Year	Deaths per Year

Breast	271,270	42,260
Lung and bronchus	228,150	142,670
Prostate	174,650	31,620
Colon and rectum	145,600	51,020
Melanoma	96,480	7,230
Lymphoma	82,310	20,970
Bladder	80,470	17,670
Kidney	73,820	14,770
Uterus	61,880	12,160
Leukemia	61,780	22,840
Pancreas	56,770	45,750
Oral cavity and pharynx	53,000	10,860
Thyroid	52,070	2,170
Liver	42,030	31,780
Myeloma	32,110	12,960

Stomach	27,510	11,140
Brain and nervous system	23,820	17,760
Ovary	22,530	13,980
Esophagus	17,650	16,080
Uterine cervix	13,170	4,250
Cancers of soft tissues including heart	12,750	5,270
Larynx	12,410	3,760
All cancers	1,762,450	606,880

Data from American Cancer Society, *Cancer Facts and Figures, 2019* (Atlanta: American Cancer Society, 2019), p. 4.

Tumor Formation

Normal cells grow, divide, mature, and die in response to a complex set of internal and external signals. A normal cell receives both stimulatory and inhibitory signals, and its growth and division are regulated by a delicate balance between these opposing forces. In a cancer cell, one or more of these signals has been disrupted, which causes the cell to proliferate at an abnormally high rate. As they lose

their response to the normal controls, cancer cells gradually lose their regular shape and boundaries, eventually forming a distinct mass of abnormal cells—a tumor. If the tumor cells remain localized, the tumor is said to be benign; if the cells invade other tissues, the tumor is said to be **malignant**. Cells that travel to other sites in the body, where they establish secondary tumors, have undergone **metastasis**.

Cancer as a Genetic Disease

Cancer arises as a result of fundamental defects in the regulation of cell division, and its study therefore has significance not only for public health but also for our basic understanding of cell biology. Through the years, many ideas have been put forth to explain cancer, but we now recognize that most, if not all, cancers arise from defects in DNA.

EVIDENCE FOR THE GENETIC THEORY OF CANCER

Early observations suggested that cancer might result from genetic damage. First, many agents that cause mutations, such as ionizing radiation and chemicals, also cause cancer (that is, they are carcinogens; see Section 18.3). Second, some cancers are consistently associated with particular chromosome abnormalities. About 90% of people with chronic myeloid leukemia, for example,

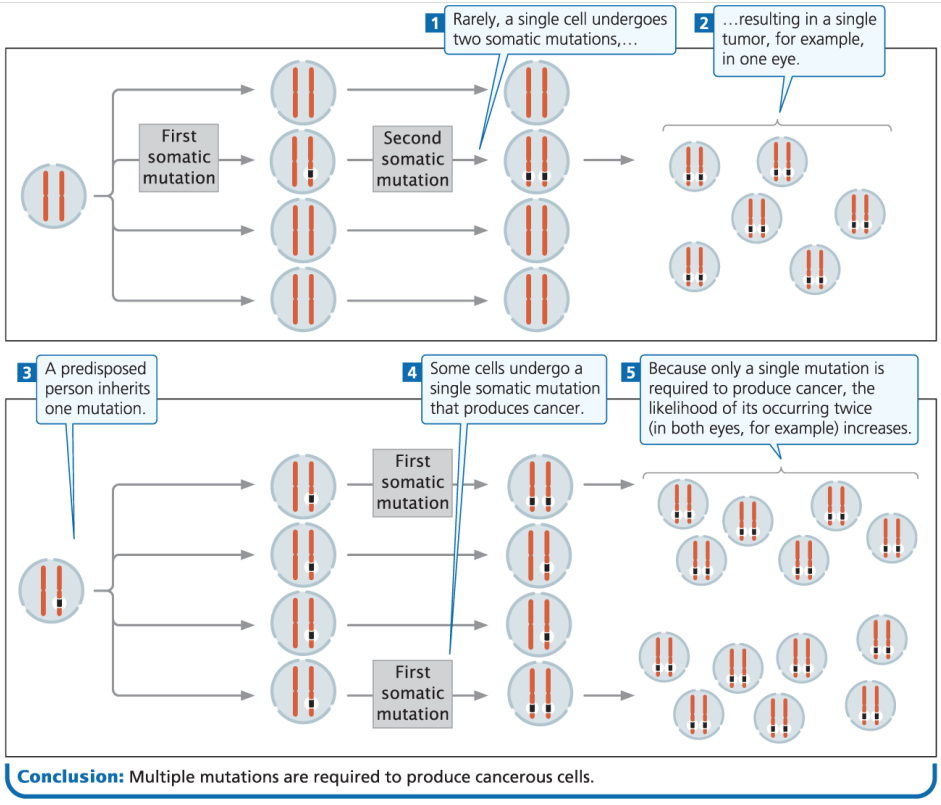
have a reciprocal translocation between chromosome 22 and chromosome 9. Third, some specific types of cancers tend to run in families. Retinoblastoma, a rare childhood cancer of the retina, appears with high frequency in a few families, in which it is inherited as an autosomal dominant trait, suggesting that a single gene is responsible for these cases of the disease.

Although these observations hinted that genes play some role in cancer, the theory of cancer as a genetic disease had several significant problems. If cancer is inherited, then every cell in the body should receive the cancer-causing gene, and therefore, every cell should become cancerous. In the types of cancer that run in families, however, tumors typically appear only in certain tissues, and often only when the person reaches an advanced age. Finally, many cancers do not run in families at all, and even in those cancers that generally do, isolated cases crop up in families with no history of the disease.

KNUDSON'S MULTISTEP MODEL OF CANCER

In 1971, Alfred Knudson proposed a model to explain the genetic basis of cancer. Knudson was studying retinoblastoma, which usually develops in only one eye but occasionally appears in both. Knudson found that when retinoblastoma appears in both eyes, onset is at an early age, and that many children with bilateral retinoblastoma have close relatives who also have the disease.

Knudson proposed that retinoblastoma results from two separate genetic defects, both of which are necessary for cancer to develop (**Figure 23.3**). He suggested that in the cases in which the disease affects just one eye, a single cell in one eye undergoes two successive mutations. Because the chance of these two mutations occurring in the same cell is remote, retinoblastoma is rare and typically develops in only one eye. Knudson proposed that children with bilateral retinoblastoma inherit one of the two mutations required for the cancer, and so every cell contains this initial mutation. In these cases, all that is required for cancer to develop is for one eye cell to undergo the second mutation. Because each eye possesses millions of cells, the probability that the second mutation will occur in at least one cell of each eye is high, so tumors may occur in both eyes at an early age.



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23.3 Alfred Knudson proposed that retinoblastoma results from two separate genetic defects, both of which are necessary for cancer to develop.

Description

The first part shows a cell with two linear chromosomes, which leads to four such cells. In the second cell, first somatic mutation occurs and one chromosome shows a change at one spot. The second somatic mutation occurs in the same cell and the other chromosome alters too. This further leads to six such cells with mutations. A caption reads, “Rarely, a single cell undergoes two somatic mutations, resulting in a single tumor, for example, in one eye.” The second part starts with a cell with mutation at one spot in one chromosome. It leads to four such cells. The first mutation occurs in two cells and their second chromosome alters too. A caption reads, “Some cells undergo a single somatic mutation that produces cancer.” Both these cells produce six mutated cells each. A caption reads, “Because only a single mutation is required to produce cancer, the likelihood of its occurring twice (in both eyes, for example) increases.” Text at the bottom reads, “Conclusion: Multiple mutations are required to produce cancerous cells.”

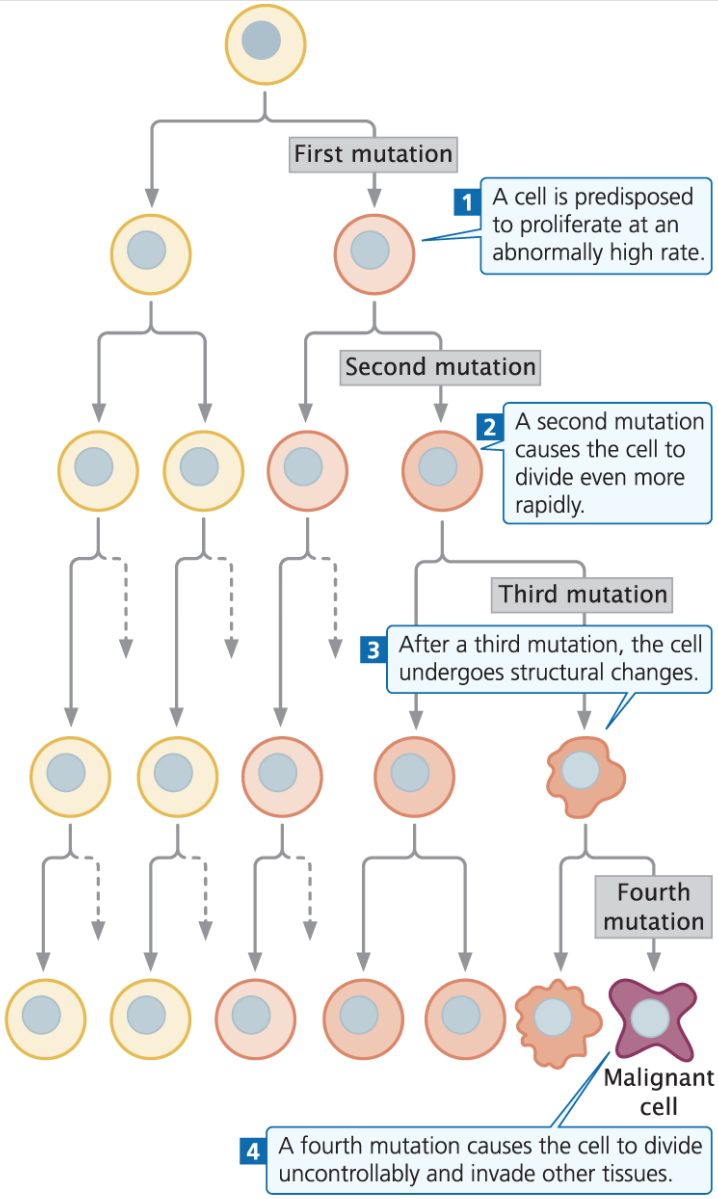
Knudson's proposal suggested that cancer is the result of a multistep process that requires several mutations. If one or more of the required mutations are inherited, fewer additional mutations are required to produce cancer, and the cancer tends to run in families. Knudson's idea has been called the "two-hit hypothesis" because, in retinoblastoma, only two mutations are necessary to cause a tumor. In most cancers, however, more than two mutations are involved in the transformation of normal cells into cancer cells. In the case of retinoblastoma, the two required mutations occur at the same locus (both alleles become mutated), but mutations at different loci are required for the development of many other cancers. The idea that cancer results from multiple mutations turns out to be correct for most cancers.

Knudson's genetic theory of cancer has been confirmed by the identification of genes that, when mutated, cause cancer. Today, we recognize that cancer is fundamentally a genetic disease, although few cancers are actually inherited. Most tumors arise from somatic mutations that accumulate over a person's life span, either through spontaneous mutation or in response to environmental mutagens.

THINK-PAIR-SHARE Question 2

THE CLONAL EVOLUTION OF TUMORS

Cancer begins when a single somatic cell undergoes a mutation that causes the cell to divide at an abnormally rapid rate. The cell proliferates, giving rise to a clone of cells, each of which carries the same mutation. Because the cells of the clone divide more rapidly than normal, they soon outgrow other cells. An additional somatic mutation that arises in some of the clone's cells may further enhance the ability of those cells to proliferate, and cells carrying both mutations soon become the most common cells in the clone. Eventually, they may be overtaken by cells that contain yet more mutations that enhance proliferation. In this process, called **clonal evolution**, the tumor cells accumulate somatic mutations that allow them to become increasingly more aggressive in their proliferative properties (**Figure 23.4**).



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23.4 Through clonal evolution, tumor cells acquire multiple mutations that allow them to become increasingly more aggressive and proliferative. To conserve space, a dashed arrow is used to represent a second cell of the same type in each case.

Description

The illustration starts with a cell and shows two branches. The first branch has the same cell. The second branch is labeled “first mutation” and shows red colored cell. A caption

reads, “A cell is predisposed to proliferate at an abnormally high rate.” The first branch produces a normal cell in every cycle. The red cell further divides into two parts; in one branch, it shows the same cell and in the second branch labeled second mutation, it shows an enlarged nucleus. A caption reads, “A second mutation causes the cell to divide even more rapidly.” Similarly, third and fourth mutations occur and the cell becomes abnormal in shape and further becomes malignant, respectively. Two captions read, “After a third mutation, the cell undergoes structural changes.” and “A fourth mutation causes the cell to divide uncontrollably and invade other tissues.”

The rate of clonal evolution depends on the frequency with which new mutations arise. Any genetic defect that allows more mutations to arise will accelerate cancer progression. Genes that regulate DNA repair are often found to have been mutated in the cells of advanced cancers, and inherited disorders of DNA repair are usually characterized by increased incidences of cancer. Because DNA-repair mechanisms normally eliminate many of the mutations that arise, cells with defective DNA-repair systems are more likely to retain mutations than are normal cells, including mutations in genes that regulate cell division. Xeroderma pigmentosum, for example, is a rare disorder caused by a defect in DNA repair (see Section 18.5). People with this condition have elevated rates of skin cancer when exposed to sunlight (which induces mutation). Similarly, breast cancer can be caused by mutations in *BRCA1* and *BRCA2*, two genes that function in DNA repair.

Mutations in genes that affect chromosome segregation may also contribute to the clonal evolution of tumors. Many cancer cells are aneuploid (they contain extra or missing copies of individual

chromosomes; see Chapter 8), and clearly, chromosome mutations can contribute to cancer progression by duplicating some genes (those on extra chromosomes) and eliminating others (those on deleted chromosomes). Cellular defects that interfere with chromosome separation increase aneuploidy and may therefore accelerate cancer progression.

CONCEPTS

Cancer is fundamentally a genetic disease. Mutations in several genes are usually required to produce cancer. If one of those mutations is inherited, fewer somatic mutations are necessary for cancer to develop, and the person may have a predisposition to cancer. Clonal evolution is the accumulation of mutations in a clone of cells.

✔ CONCEPT CHECK 1

How does the multistep model of cancer explain the observation that sporadic cases of retinoblastoma usually appear in only one eye, whereas inherited forms of the cancer appear in both eyes?

The Role of Environmental Factors in Cancer

Although cancer is a genetic disease, most cancers are not inherited, and many are influenced by environmental factors. The role of environmental factors in cancer is suggested by differences in the

incidence of specific cancers throughout the world (Table 23.2). The results of studies show that migrant populations typically take on the cancer incidence of their host country. For example, the overall rates of colon cancer are considerably lower in Japan than in Hawaii. However, within a single generation after migration to Hawaii, Japanese people develop colon cancer at rates similar to or exceeding those of Caucasian Hawaiians. The increased cancer rate among the migrants is due to the fact that they are exposed to the same environmental factors as the natives are.

TABLE 23.2 Examples of geographic variation in the incidence of cancer

Type of Cancer	Location	Incidence Rate*
Lip	Canada (Newfoundland)	15.1
	Brazil (Fortaleza)	1.2
Nasopharynx	Hong Kong	30.0
	United States (Utah)	0.5
Colon	United States (Iowa)	30.1
	India (Mumbai)	3.4
Lung	United States (New Orleans,	110.0

	African Americans)	
	Costa Rica	17.8
Prostate	United States (Utah)	70.2
	China (Shanghai)	1.8
Bladder	United States (Connecticut)	25.2
	Philippines (Rizal)	2.8
All cancer	Switzerland (Basel)	383.3
	Kuwait	76.3

Data from C. Muir et al., *Cancer Incidence in Five Continents*, vol. 5 (Lyon: International Agency for Research on Cancer, 1987), Table 12-2.
*The incidence rate is the age-standardized rate in males per 100,000 population.

A number of environmental factors contribute to cancer, but those that have the greatest effects include tobacco use, diet, obesity, alcohol, and UV radiation (**Table 23.3**). Other environmental factors that induce cancer are certain types of chemicals, such as benzene (used as an industrial solvent), benzo[a]pyrene (found in cigarette smoke), and polychlorinated biphenyls (PCBs; used in industrial transformers and capacitors). Most environmental factors associated with cancer cause somatic mutations that stimulate cell division or otherwise affect the process of cancer progression. Age, although not an environmental factor in the usual sense, is a risk factor for

many cancers, with the chance of developing cancer increasing with age.

THINK-PAIR-SHARE Question 3

TABLE 23.3 Percentage of cancer cases in the United Kingdom attributed to environmental factors

Factor	Percentage of Cancer Cases
Tobacco	19.4
Diet	9.2
Overweight and obesity	5.5
Alcohol	4.0
Occupation	3.7
Radiation (UV)	3.5
Infections	3.1
Radiation (ionizing)	1.8
All environmental factors	42.7

Data from D. M. Parkin, L. Boyd, and L. C. Walker. 2011. Fraction of cancer attributable to lifestyle and environmental factors in the UK in 2010. *British Journal of Cancer* 105:S77–S81.

Environmental factors may interact with genetic predispositions to cancer. Lung cancer, for example, is clearly associated with smoking, an environmental factor. Genome-wide association studies (see Section 20.1) revealed that variation at several genes predisposes some people to smoking-induced lung cancer. Variants at some of these genes cause people to be more likely to become addicted to smoking. Other predisposing genes encode receptors that bind potential carcinogens in cigarette smoke. › **TRY PROBLEM 23**

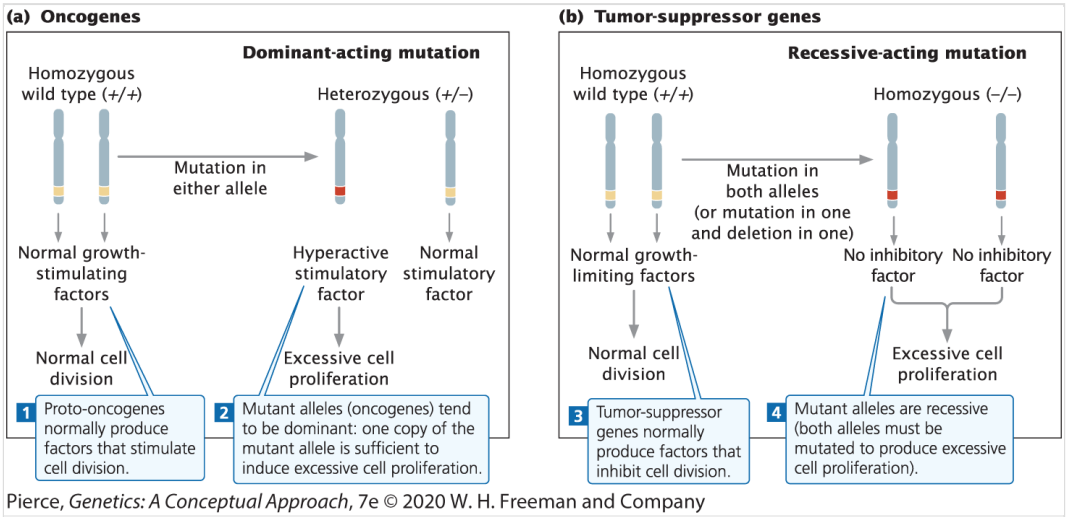
23.2 Mutations in Several Types of Genes Contribute to Cancer

As we have learned, cancer is a disease caused by alterations in DNA. However, there are many different types of genetic alterations that may contribute to the development of cancer. More than 350 different human genes have been identified that contribute to cancer; the actual number is probably much higher. Research on mice suggests that more than 2000 genes can, when mutated, contribute to the development of cancer. In this section, we consider some of the different types of genes that frequently have roles in cancer.

Oncogenes and Tumor-Suppressor Genes

The signals that regulate cell division fall into two basic types: molecules that stimulate cell division and molecules that inhibit it. These control mechanisms are similar to the accelerator and brake of a car. In normal cells (but, one would hope, not in your car), both accelerators and brakes are applied at the same time, causing cell division to proceed at the proper speed.

Because cell division is affected by both accelerators and brakes, cancer can arise from mutations in either type of signal, so there are several fundamentally different routes to cancer. A stimulatory gene can be made hyperactive or active at inappropriate times, which is analogous to having a car's accelerator stuck in the floored position. Mutations in stimulatory genes usually act in a dominant manner because even the amount of gene product produced by a single allele is usually sufficient to have a stimulatory effect. Mutated dominant-acting stimulatory genes that cause cancer are termed **oncogenes** (**Figure 23.5a**).



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23.5 Both oncogenes and tumor-suppressor genes contribute to cancer, but they differ in their modes of action and dominance.

Description

Illustration "a" shows oncogenes. It shows 2 sister chromosomes labeled homozygous wild type (positive over positive), each with an oncogene. Normal growth stimulating factors lead these chromosomes to normal cell division. A caption reads, "Proto-oncogenes normally produce factors that stimulate cell division." Mutation in either allele occurs and produces heterozygous (positive over negative). One chromosome leads to

normal stimulatory factor and the other with hyperactive stimulatory factor leads to excessive cell proliferation. A caption reads, “Mutant alleles (oncogenes) tend to be dominant: one copy of the mutant allele is suffi¬cient to induce excessive cell proliferation.”

Illustration “b” shows tumor-suppressor genes. It shows 2 sister chromosomes labeled homozygous wild type (positive over positive), each with an oncogene. Normal growth limiting factors lead these chromosomes to normal cell division. A caption reads, “Tumor-suppressor genes normally produce factors that inhibit cell division.” Mutation in both alleles (or mutation in one and deletion in one) occurs and produces homozygous (negative over negative). Both the chromosomes lead to excessive cell proliferation through no inhibitory factor. A caption reads, “Mutant alleles are recessive (both alleles must be mutated to produce excessive cell proliferation).”

Cell division may also be stimulated when inhibitory genes are made *inactive*, which is analogous to having a defective brake in a car. Mutated inhibitory genes generally act in a recessive manner because both copies must be mutated to remove all inhibition. Inhibitory genes involved in cancer are termed **tumor-suppressor genes** (**Figure 23.5b**). Many cancer cells have mutations in both oncogenes and tumor-suppressor genes.

Although oncogenes or mutated tumor-suppressor genes, or both, are required to produce cancer, mutations in DNA-repair genes can increase the likelihood of acquiring mutations in those genes. Having mutated DNA-repair genes is analogous to having a lousy car mechanic who does not make the necessary repairs on a broken accelerator or brake.

ONCOGENES

Oncogenes were the first cancer-causing genes to be identified. In 1909, a farmer brought physician Peyton Rous a hen with a large connective-tissue tumor (sarcoma) growing on its breast. When Rous injected pieces of this tumor into other hens, they also developed sarcomas. Rous conducted experiments that demonstrated that the tumors were being transmitted by a retrovirus, which became known as the Rous sarcoma virus. A number of other cancer-causing viruses were subsequently isolated from various animal tissues. These viruses were generally assumed to carry a cancer-causing gene that was transferred to the host cell. The first oncogene, called *src*, was isolated from the Rous sarcoma virus in 1970.

In 1975, Michael Bishop, Harold Varmus, and their colleagues began to use probes (see Chapter 19) for viral oncogenes to search for related sequences in normal cells. They discovered that the genomes of all normal cells carry DNA sequences that are closely related to oncogenes. These normal cellular genes are called **proto-oncogenes**. They are responsible for basic cellular functions in normal cells, but when mutated, they become oncogenes that contribute to the development of cancer. When a virus infects a cell, a proto-oncogene may become incorporated into the viral genome through recombination. Within the viral genome, the proto-oncogene may mutate to an oncogene that, when inserted back into a host cell, causes rapid cell division and cancer. While viruses are capable of converting proto-oncogenes into oncogenes, most proto-oncogenes are mutated to form oncogenes without the involvement of a virus.

Many oncogenes have been identified by experiments in which selected fragments of DNA are added to cells in culture. Some of the cells take up the DNA, and if these cells become cancerous, then the DNA fragment that was added to the culture must contain an oncogene. The fragments can then be sequenced and the oncogene identified. A large number of oncogenes have now been discovered (Table 23.4). About 90% of all cancer genes are thought to be dominant oncogenes.

TABLE 23.4 Some oncogenes and functions of their corresponding proto-oncogenes

Gene	Normal Function	Cancer in Which Gene Is Mutated
erbB	Part of growth factor receptor	Many types of cancer
fos	Transcription factor	Osteosarcoma and endometrial carcinoma
jun	Transcription factor, cell cycle control	Lung cancer, breast cancer
myc	Transcription factor	Lymphomas, leukemias, neuroblastoma
ras	GTP binding and GTPase	Many types of cancer

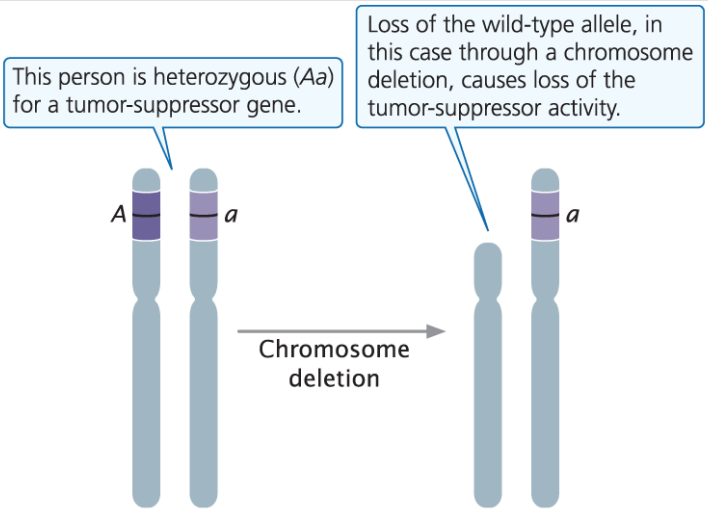
sis	Growth factor	Glioblastomas and other cancers
src	Protein tyrosine kinase	Many types of cancer

TUMOR-SUPPRESSOR GENES

Tumor-suppressor genes are more difficult to identify than oncogenes because they *inhibit* cancer and are recessive; both alleles must be mutated before the inhibition of cell division is removed. Because the *failure* of their function promotes cell proliferation, tumor-suppressor genes cannot be identified by adding them to cells and looking for cancer. About 10% of cancer-causing genes are thought to be tumor-suppressor genes, but their impact is substantial: most cancers contain one or more mutated tumor-suppressor genes.

Defects in both copies of a tumor-suppressor gene are usually required to cause cancer. An organism can inherit one defective copy of a tumor-suppressor gene (heterozygous for a cancer-causing mutation) and not have cancer, because the remaining normal allele produces the tumor-suppressing product. However, these heterozygotes are often predisposed to cancer because the inactivation or loss of the one remaining normal allele is all that is required to completely eliminate the tumor-suppressor gene

product. Inactivation of the remaining wild-type allele of a heterozygote is referred to as the **loss of heterozygosity**. A common mechanism for the loss of heterozygosity is a deletion on the chromosome that carried the normal copy of the tumor-suppressor gene (Figure 23.6). Sometimes, however, mutations in a tumor-suppressor gene can act in a dominant fashion; this may occur when the mutation alters the sequence or expression of the tumor-suppressor protein in such a way that it gains a new function that contributes to cancer.



23.6 The loss of heterozygosity often leads to cancer in a person heterozygous for a tumor-suppressor gene.

Description

The illustration shows two chromosomes of equal size; one with big A and the other with small A allele. A caption reads, “This person is heterozygous (big A small A) for a tumor-suppressor gene.” The chromosome with big A allele loses its allele and the other remains

the same. A caption reads, “Loss of the wild-type allele, in this case through a chromosome deletion, causes loss of the tumor-suppressor activity.” Text at the bottom reads, “Conclusion: People heterozygous for a tumor-suppressor gene are predisposed to cancer.”

Among the first tumor-suppressor genes to be identified was the gene that causes retinoblastoma. In 1985, Raymond White and Webster Cavenne showed that large segments of chromosome 13 were missing in cells from retinoblastoma tumors, and later, the *RB* tumor-suppressor gene was isolated from those segments. Another example of a tumor-suppressor gene is *BRCA1*, mutations of which are associated with increased risk of breast and ovarian cancer. *BRCA1* produces a protein that normally helps in the repair of double-strand breaks in DNA by homology directed repair (see Section 18.5). The protein also acts as a transcription factor and interacts with histone deacetylase enzymes, which affect transcription. A number of tumor-suppressor genes have now been discovered (**Table 23.5**).

TABLE 23.5 Some tumor-suppressor genes and their normal functions

Gene	Normal Function	Cancer in Which Gene Is Mutated
APC	Scaffold protein, interacts with microtubules	Colorectal

BRCA1	DNA repair, transcription factor	Breast and ovarian
CDKN2A	Regulates cell division	Melanoma
NF1	GTPase activator	Neurofibromatosis
p53	Regulates cell division, apoptosis, DNA repair, and other functions	Many types of cancer
RB	Regulates cell division	Retinoblastoma and many other cancers

Sometimes the mutation or loss of a single allele of a tumor-suppressor gene is sufficient to cause cancer. This effect—the appearance of an abnormal trait in an individual cell or organism that is heterozygous for that trait—is called **haploinsufficiency**. This phenomenon is thought to be due to dosage effects: the heterozygote, with only one functional allele, usually produces only half as much of the product encoded by the tumor-suppressing gene as the homozygote does, and this amount is insufficient to prevent tumor formation. To better understand the difference between oncogenes and tumor suppressor genes, see (A) **Animation 23.1. ›**

TRY PROBLEM 24

CONCEPTS

Proto-oncogenes are genes that control normal cellular functions; when mutated, they become oncogenes that stimulate cell proliferation. They tend to be dominant in their action. Tumor-suppressor genes normally inhibit cell proliferation; when mutated, they allow cells to proliferate. Tumor-suppressor genes tend to be recessive in their action. Individual organisms that are heterozygous for tumor-suppressor genes are often predisposed to cancer.

✔ CONCEPT CHECK 2

Why are oncogenes usually dominant in their action, whereas tumor-suppressor genes are recessive?

Genes That Control the Cell Cycle

The cell cycle is the normal process by which cells undergo growth and division. Normally, progression through the cell cycle is tightly regulated so that cells divide only when additional cells are needed, when all the components necessary for division are present, and when the DNA has been replicated without damage. Sometimes, however, errors arise in one or more of the components that regulate the cell cycle. These errors often cause cells to divide at inappropriate times or rates, leading to cancer. Indeed, many proto-oncogenes and tumor-suppressor genes function normally by helping to control the cell cycle. Before considering how errors in

this system contribute to cancer, we must first understand how the cell cycle is usually regulated.

CONTROL OF THE CELL CYCLE

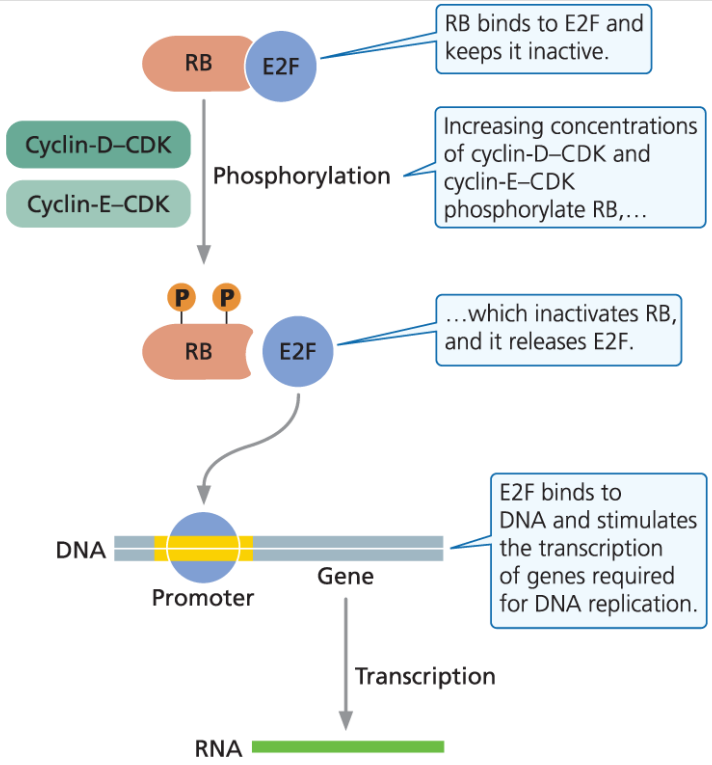
As discussed in Chapter 2, the cell cycle is the series of stages through which a cell passes from one cell division to the next. Cells that are actively dividing pass through the G_1, S, and G_2 phases of interphase and then move directly into the M phase, in which cell division takes place. Nondividing cells pass from G_1 into the G_0 stage, in which they are functional but not actively growing or dividing. Progression from one stage of the cell cycle to another is influenced by a number of internal and external signals and is regulated at key points in the cycle, called checkpoints.

Key events of the cell cycle are controlled by **cyclin-dependent kinases (CDKs)**. Kinases are enzymes that phosphorylate (add phosphate groups to) other proteins. In some cases, phosphorylation activates the other protein; in others, it inactivates the other protein. As their name implies, CDKs are functional only when associated with another type of protein, called a **cyclin**. The levels of cyclins oscillate over the course of the cell cycle; when bound to a CDK, a cyclin specifies which proteins the CDK will phosphorylate. Each cyclin appears at a specific point in the cell cycle, usually because its synthesis and destruction are regulated by another cyclin. Cyclins and CDKs are called by different names in

different organisms; here, we use the terms applied to these molecules in mammals.

G_1-TO-S TRANSITION

Let's begin by looking at the G_1-to-S transition. As mentioned earlier, cell cycle checkpoints ensure that all cellular components are present and in good working order before the cell proceeds to the next stage of the cycle. The G_1/S checkpoint is at the end of G_1, just before the cell enters the S phase and replicates its DNA. The cell is prevented from passing through the G_1/S checkpoint by the retinoblastoma (RB) protein (**Figure 23.7**), which binds to a transcription factor called E2F and keeps it inactive. During G_1, cyclin D and cyclin E continuously increase in concentration and combine with their associated CDKs. Cyclin-D–CDK and cyclin-E–CDK both phosphorylate molecules of RB. By late in G_1, the phosphorylation of RB is completed, which inactivates RB. Without the inhibitory effects of RB, E2F is released. The E2F transcription factor stimulates the transcription of genes that produce enzymes necessary for the replication of DNA, and the cell moves into the S phase of the cell cycle.



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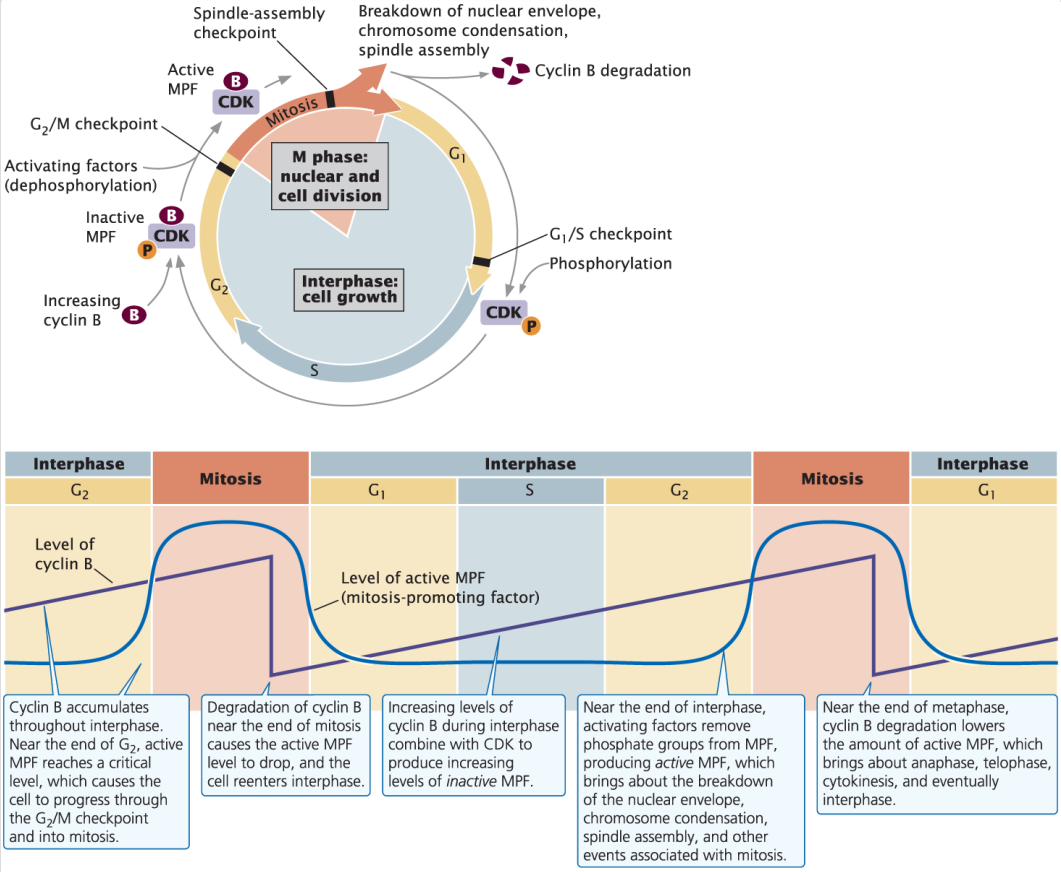
23.7 The RB protein helps control a cell's progression through the G_1/S checkpoint by binding transcription factor E2F.

Description

R B binds to E 2 F and phosphorylation occurs in the presence of cyclin-D-C D K and cyclin-E-C D K. A caption reads, "R B binds E 2 F and keeps it inactive." Two P attaches to R B and E 2 F complex. A caption reads, "Increasing concentrations of cyclin-D–C D K and cyclin-E–C D K phosphorylate R B, which inactivates R B, and it releases E 2 F." The E 2 F then binds to promoter in a D N A. From which, a gene is transcribed and R N A is made. A caption reads, "E 2 F binds to D N A and stimulates the transcription of genes required for D N A replication."

G_2-TO-M TRANSITION

Regulation of the G_2-to-M transition is similar to that of the G_1-to-S transition. In the G_2-to-M transition, cyclin B combines with a CDK to form an inactive complex called *mitosis-promoting factor* (MPF). After MPF has been formed, it must be activated by the removal of a phosphate group (Figure 23.8). During G_1, cyclin B levels are low, so the amount of MPF is also low. As more cyclin B is produced, it combines with CDK to form increasing amounts of MPF. Near the end of G_2, the amount of active MPF reaches a critical level, which commits the cell to divide. The MPF concentration continues to increase, reaching a peak in mitosis.



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23.8 Progression through the G_2/M checkpoint is regulated by cyclin B.

Description
The illustration starts with a cell cycle. The G 1 phase begins on the top right side. Below this is the G 1-S checkpoint, where C-D-K gets phosphorylated. The S phase passes into the G 2 phase shown on the bottom left. Increasing cyclin B, an inactive M-P-F, and activating factors are shown. At the 10 o'clock point is the G 2-M checkpoint and the beginning of mitosis. An active C D K and the spindle-assembly checkpoint are present. At the end of mitosis, the breakdown of nuclear envelope, chromosome condensation, spindle assembly, and cyclin B degradation occurs. Below the cell cycle, a table with five columns and two rows is shown. The column headers are as follows: Interphase further subdivided into G 2; mitosis; interphase further subdivided into G 1, S, and G 2; mitosis; and interphase further subdivided into G 1. The table shows two curves for level of cyclin B and level of active M P F (mitosis-promoting factor). Level of cyclin B rises in first column, continues to rise in column 2 and declines steeply in the same column. A caption reads, "Cyclin B accumulates throughout interphase. Near the end of G 2, active M P F reaches a critical level, which causes the cell to progress through the G 2 over M checkpoint and into mitosis." Another caption reads, "Degradation of cyclin B near the end of mitosis causes the active M P F level to drop, and the cell reenters interphase." Then, rises till column 4 and declines again in the same column and rises in the last column. A caption reads, "Increasing levels of cyclin B during interphase combine with C D K to produce increasing levels of inactive M P F." Level of active M P F (mitosis-promoting factor) starts at a low level in column 2, forms a high concave down curve in column 2, declines in column 3, forms another high concave down curve in column 4, and declines again in column 5. Two captions read as follows: "Near the end of interphase, activating factors remove phosphate groups from M P F, producing active M P F, which brings about the breakdown of the nuclear envelope, chromosome condensation, spindle assembly, and other events associated with mitosis. Near the end of metaphase, cyclin B degradation lowers the amount of active M P F, which brings about anaphase, telophase, cytokinesis, and eventually interphase."

The active form of MPF phosphorylates other proteins, which then bring about many of the events associated with mitosis, such as nuclear-membrane breakdown, mitotic spindle formation, and chromosome condensation. At the end of metaphase, cyclin B is

abruptly degraded, which lowers the amount of MPF and, by initiating anaphase, sets in motion a chain of events that ultimately brings mitosis to a close (**Figure 23.8**). In brief, high levels of active MPF stimulate mitosis, and low levels of MPF bring a return to interphase conditions.

The G_2/M checkpoint is at the end of G_2, before the cell enters mitosis. A number of factors stimulate the synthesis of cyclin B and the activation of MPF, whereas other factors inhibit MPF. Together, these factors ensure that mitosis is not initiated until conditions are appropriate for cell division. For example, DNA damage inhibits the activation of MPF; consequently, the cell is arrested in G_2 and does not undergo division.

SPINDLE-ASSEMBLY CHECKPOINT

Yet another checkpoint, called the spindle-assembly checkpoint, functions in metaphase. This checkpoint delays the onset of anaphase until all chromosomes are aligned on the metaphase plate and sister kinetochores are attached to spindle microtubules from opposite poles. If all chromosomes are not properly aligned, the checkpoint blocks the destruction of cyclin B. The persistence of cyclin B keeps MPF active and maintains the cell in a mitotic state. An additional checkpoint controls the cell's exit from mitosis.

MUTATIONS IN CELL CYCLE CONTROL AND CANCER

Many cancers are caused by defects in the cell cycle's regulatory machinery. For example, mutations in the gene that encodes the RB protein—which normally holds the cell in G_1 until the DNA is ready to be replicated—are associated with many cancers, including retinoblastoma. When the *RB* gene is mutated, cells pass through the G_1/S checkpoint without the normal controls that prevent cell proliferation. The gene that encodes cyclin D (which stimulates the passage of cells through the G_1/S checkpoint) is overexpressed in about 50% of all breast cancers, as well as in some cases of esophageal and skin cancer. Likewise, the tumor-suppressor gene *p53* (also known as *TP53*), which is mutated in about 75% of all colon cancers, regulates a potent inhibitor of CDK activity.

Some proto-oncogenes and tumor-suppressor genes have roles in apoptosis, a process of programmed cell death in which the cell's DNA is degraded, its nucleus and cytoplasm shrink, and the cell undergoes phagocytosis by other cells without the leakage of its contents. Each cell has the ability to assess its own state, and if it detects abnormalities or damage, they normally undergo apoptosis (see Section 22.4). Cancer cells frequently have chromosome mutations, DNA damage, or other cellular anomalies that would normally stimulate apoptosis and prevent their proliferation. Many of these cells have mutations in genes that regulate apoptosis and therefore do not undergo programmed cell death. The ability of a cell to initiate apoptosis in response to DNA damage, for example, depends on *p53*, which is inactive in many human cancers.

Another cellular process that plays a role in cancer is autophagy, in which proteins, cytoplasm, damaged organelles, and other cellular components are engulfed by vesicles within the cell and transported to lysosomes, where they are degraded and their components recycled. Autophagy maintains the quality of the proteins and organelles within the cell and helps during times of stress by recycling essential components and conserving energy. Autophagy has been shown to suppress tumor formation under some conditions and promote tumor growth under others. For example, mice that are deficient in autophagy show elevated rates of cancer because autophagy maintains cellular homeostasis and maintains organelle quality, both of which help prevent cancer development. On the other hand, autophagy can promote cancer by allowing cancer cells to survive the stressful conditions of tumor development and cancer treatment. Thus, with regard to cancer, autophagy has been called both friend and foe. A number of proto-oncogenes and tumor-suppressor genes affect the process of autophagy. For example, mutations in *p53* alter autophagy in ways that contribute to cancer development.

CONCEPTS

Progression through the cell cycle is controlled at checkpoints, which are regulated by interactions between cyclins and cyclin-dependent kinases. Genes that control the cell cycle are frequently mutated in cancer cells. Apoptosis and autophagy also influence cancer development, and genes that affect these processes are frequently mutated in cancer cells.

✔ CONCEPT CHECK 3

What would be the most likely effect of a mutation that causes cyclin B to be unable to bind to CDK?

a. Cells pass through the G_2/M checkpoint and enter mitosis even when DNA has not been replicated.
b. Cells never pass through the G_1/S checkpoint.
c. Cells pass through mitosis more quickly than do nonmutated cells.
d. Cells fail to pass through the G_2/M checkpoint and do not enter mitosis.

SIGNAL-TRANSDUCTION PATHWAYS

Whether cells pass through the cell cycle and continue to divide is influenced by a large number of internal and external signals. External signals are initiated by hormones and growth factors. These molecules are often unable to pass through the cell membrane because of their size or charge; they exert their effects by binding to receptors on the cell surface. This binding triggers a series of intracellular reactions that then carry the message to the nucleus or another site within the cell. This type of system, in which an external signal triggers a cascade of intracellular reactions that ultimately produce a specific response, is called a **signal-transduction pathway**. Defects in signal-transduction pathways are often associated with cancer.

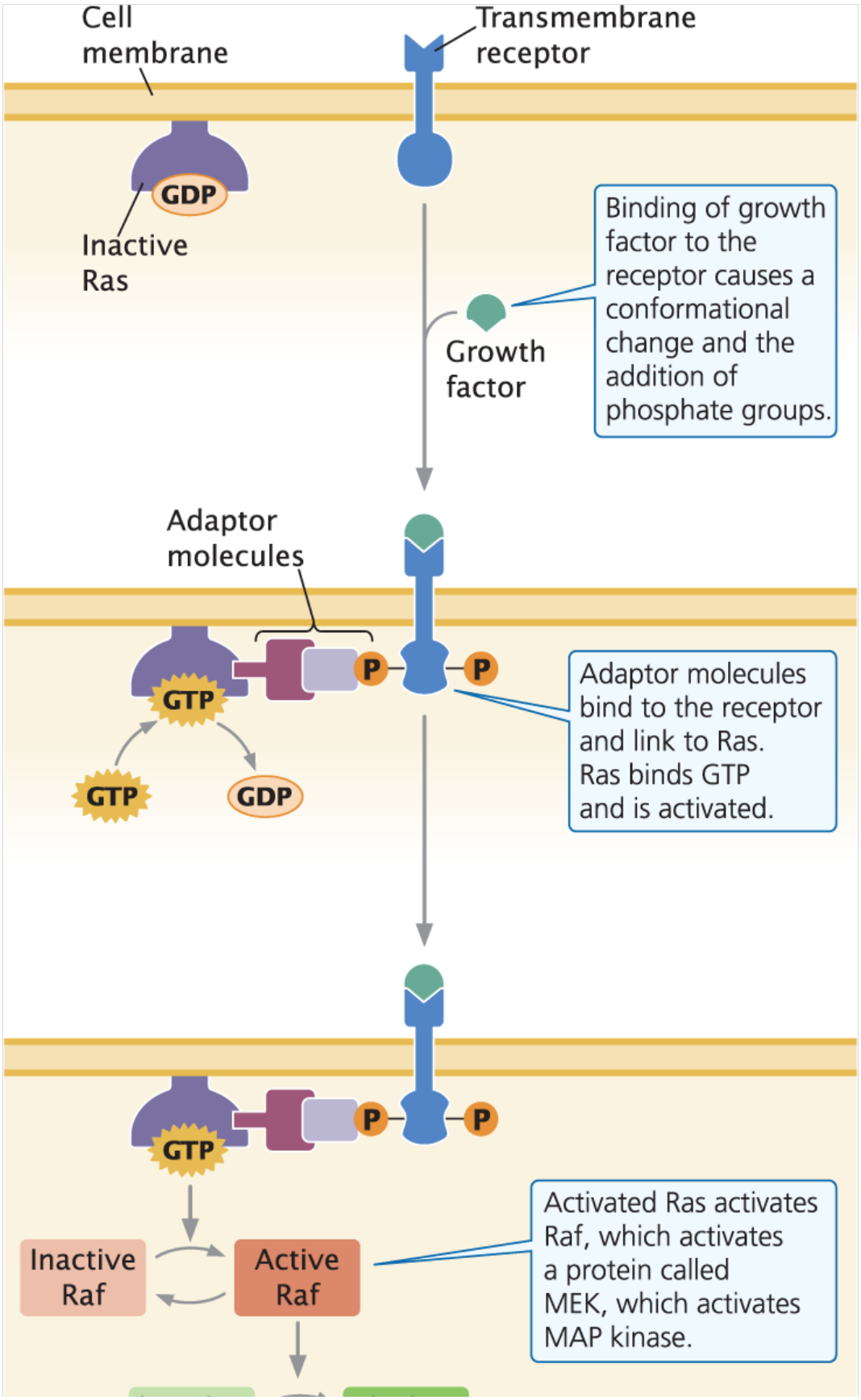
A signal-transduction pathway begins with the binding of an external signaling molecule to a specific receptor that is embedded in the cell membrane. Receptors in signal-transduction pathways usually have three parts: (1) an extracellular domain that protrudes from the cell

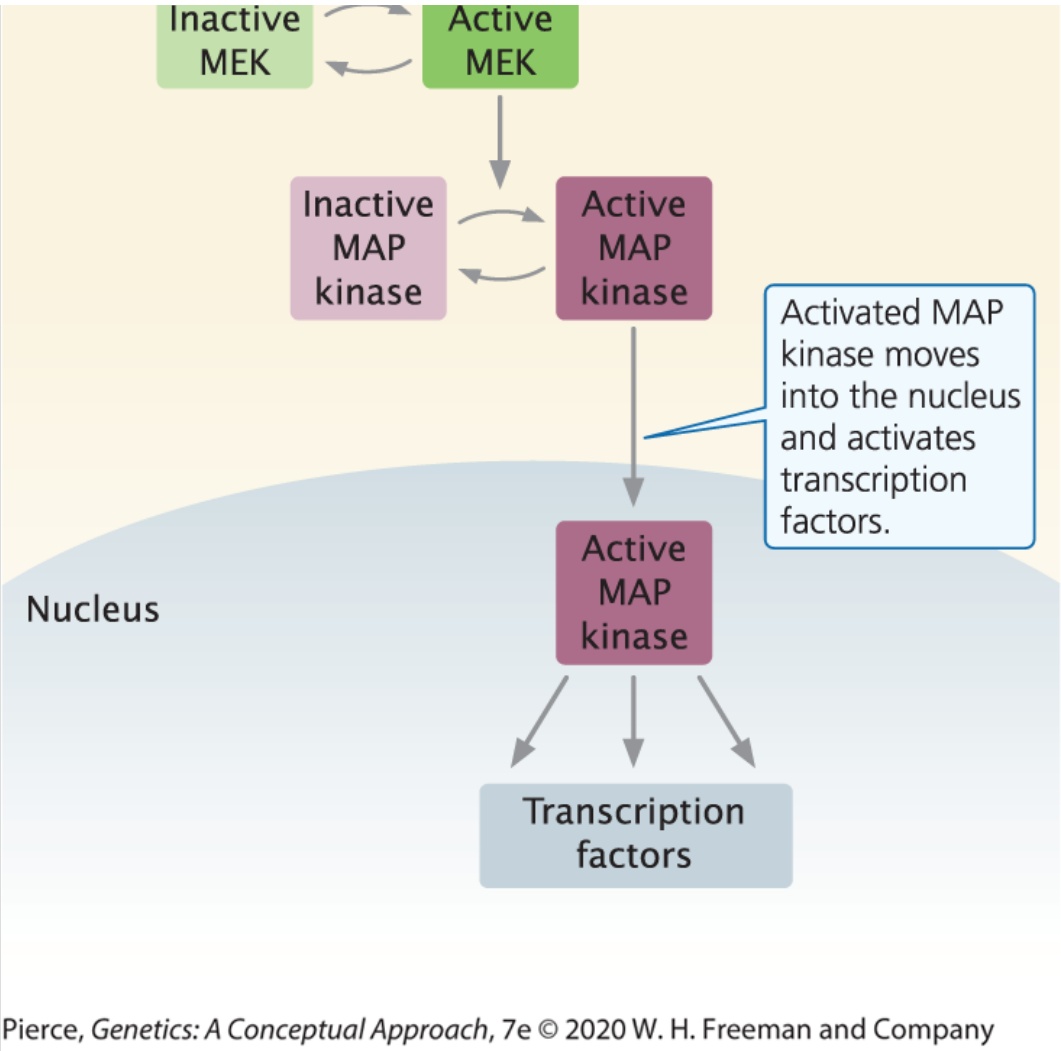
and binds the signaling molecule; (2) a transmembrane domain that passes across the membrane and conducts the signal to the interior of the cell; and (3) an intracellular domain that extends into the cytoplasm and, upon the binding of the signaling molecule, undergoes a chemical or conformational change that is transmitted to molecules of the signal-transduction pathway in the cytoplasm. The binding of a signaling molecule to the membrane-bound receptor activates a protein in the pathway. On activation, this protein activates the next molecule in the pathway, often by adding or removing phosphate groups or causing changes in the conformation of the protein. The newly activated protein activates the next molecule in the pathway, and in this way, the signal is passed along through a cascade of reactions and ultimately produces the response, such as stimulating or inhibiting the cell cycle.

Researchers have determined the pathways by which various signals influence the cell cycle. To illustrate signal transduction, let's consider the Ras signal-transduction pathway, which plays an important role in control of the cell cycle. Each Ras protein cycles between an active form and an inactive form. In its inactive form, a Ras protein is bound to guanosine diphosphate (GDP); in its active form, it is bound to guanosine triphosphate (GTP).

The Ras signal-transduction pathway is activated when a growth factor, such as epidermal growth factor (EGF), binds to a receptor on the cell membrane (**Figure 23.9**). The binding of EGF causes a conformational change in the receptor and the addition of

phosphate groups to it. The addition of the phosphate groups allows adaptor molecules to bind to the receptor. These adaptor molecules link the receptor with an inactive molecule of Ras protein. The adaptor molecules activate Ras by stimulating it to release GDP and bind GTP. The newly activated Ras protein then binds to an inactive form of another protein, called Raf, and activates it. After activating Raf, the Ras protein hydrolyzes GTP to GDP, returning to its inactive form.


Cell membrane
Transmembrane receptor
GDP
Inactive Ras
Binding of growth factor to the receptor causes a conformational change and the addition of phosphate groups.
Growth factor
Adaptor molecules
GTP
P
P
GTP
GDP
Adaptor molecules bind to the receptor and link to Ras. Ras binds GTP and is activated.
GTP
P
P
Inactive Raf
Active Raf
Activated Ras activates Raf, which activates a protein called MEK, which activates MAP kinase.



23.9 The Ras signal-transduction pathway conducts signals from growth factors and hormones to the nucleus and stimulates the cell cycle. Mutations in this pathway often contribute to cancer.

Description

The illustration shows transmembrane receptor attached to a cell membrane. Inactive Ras attached to G D P is shown on in the inner side of the membrane. A growth factor attaches to the transmembrane receptor. A caption reads, “Binding of growth factor to the receptor causes a conformational change and the addition of phosphate groups.” Adaptor molecules link the inactive Ras and transmembrane receptor with growth factor. G T P gets converted into G D P in Ras. A caption reads, “Adaptor molecules bind to the receptor and link to Ras. Ras binds G T P and is activated.” A series of reaction is activated then. A caption reads, “Activated Ras activates Raf, which activates a protein called MEK, which

activates MAP kinase." Inactive Raf converts into active Raf leading to inactive MEK converts into active MEK leading to inactive MAP kinase converts into active MAP kinase. This active MAP kinase enters the nucleus and leads to transcription factors. A caption reads, "Activated MAP kinase moves into the nucleus and activates transcription factors."

Activated Raf then sets in motion a cascade of reactions that ends in the activation of a protein called MAP kinase. Activated MAP kinase moves into the nucleus and phosphorylates a number of transcription factors, which then stimulate the transcription of genes taking part in the cell cycle. In this way, the original external signal promotes cell division. A number of other signal-transduction pathways that affect the cell cycle and cell proliferation have been identified.

Because signal-transduction pathways help control the cell cycle, defects in their components often contribute to cancer. For example, genes that encode Ras proteins are frequently oncogenes, and mutations in these genes are often found in cancer cells: 95% of tumors of the pancreas and 45% of colorectal tumors have mutations in *ras* genes. Mutations in these genes produce mutant Ras proteins that are permanently activated and continuously stimulate cell division.

CONCEPTS

Molecules outside the cell often bring about intracellular responses by binding to a transmembrane receptor and stimulating a cascade of intracellular reactions, which is known as a signal-transduction pathway. Many molecules in the pathway are proteins that alternate

between active and inactive forms. Defects in signal-transduction pathways are often associated with cancer.

✔ CONCEPT CHECK 4

Ras proteins are activated when they

a. bind GTP.
b. release GTP.
c. bind GDP.
d. undergo acetylation.

DNA-Repair Genes

As we have seen, cancer arises from the accumulation of multiple mutations in a single cell. Some cancer cells have normal rates of mutation, and multiple mutations accumulate because each mutation gives the cell a replicative advantage over cells without the mutations. Other cancer cells have higher-than-normal rates of mutation in all of their genes, which leads to more frequent mutation of oncogenes and tumor-suppressor genes. What might be the source of these high rates of mutation in some cancer cells?

Two processes control the rate at which mutations arise within a cell: (1) the rate at which errors arise during and after the course of DNA replication and (2) the efficiency with which these errors are corrected. The error rate in replication is controlled by the fidelity of DNA polymerases and other proteins involved in the replication

process (see Chapter 12). However, defects in genes encoding replication proteins have not been strongly linked to cancer.

The mutation rate is also strongly affected by whether errors are corrected by DNA-repair systems (see Section 18.5). Defects in genes that encode components of these repair systems have been consistently associated with a number of cancers. People with xeroderma pigmentosum, for example, are defective in nucleotide-excision repair, an important DNA-repair system that normally corrects DNA damage caused by a number of mutagens, including ultraviolet light. Likewise, about 13% of colorectal, endometrial, and stomach cancers have cells that are defective in mismatch repair, another major DNA-repair system in the cell.

A particular type of colon cancer, called hereditary nonpolyposis colon cancer, is inherited as an autosomal dominant trait. In families with this condition, a person can inherit one mutated and one normal allele of a gene that controls mismatch repair. The normal allele provides sufficient levels of the protein product for mismatch repair to function, but it is highly likely that this normal allele will become mutated or lost in at least a few cells. If it does so, there is no mismatch repair, and these cells undergo higher-than-normal rates of mutation, leading to defects in oncogenes and tumor-suppressor genes that cause the cells to proliferate.

Defects in DNA-repair systems may also contribute to the generation of chromosome rearrangements and genomic instability. Many DNA-

repair systems make single- and double-strand breaks in the DNA as part of the repair process. If these breaks are not repaired properly, then chromosome rearrangements often result.

Genes That Regulate Telomerase

Another factor that may contribute to the progression of cancer is the inappropriate activation of the enzyme telomerase. Telomeres are special sequences at the ends of eukaryotic chromosomes. Recall from Chapter 12 that in most somatic cells, the ends of chromosomes cannot be replicated, and the telomeres become shorter with each cell division. This shortening eventually leads to the destruction of the chromosomes and cell death, so somatic cells are capable of only a limited number of cell divisions.

In germ cells and stem cells, telomerase replicates the chromosome ends (see pp. 364–366 in Chapter 12), thereby maintaining the telomeres, but this enzyme is not normally expressed in somatic cells. In many tumor cells, however, sequences that regulate the expression of the telomerase gene are mutated, allowing the enzyme to be expressed, and the cell is capable of unlimited cell division. This mutation allows cancer cells to divide indefinitely.

Neuroblastoma, for example, is a tumor of the sympathetic nervous system that occurs in children. Many of these tumors are low-risk, regressing spontaneously or responding to treatment. On the other

hand, about half are high-risk, do not respond to treatment, and are often lethal. In an attempt to differentiate between low-risk and high-risk neuroblastoma tumors, researchers sequenced the entire genome of a series of these tumors. They found that many of the high-risk tumors had chromosome rearrangements or other mutations that increased the expression of telomerase. Thus, expression of telomerase appears to be a characteristic of many high-risk neuroblastomas.

Genes That Promote Vascularization and the Spread of Tumors

Another important group of factors that contribute to the progression of cancer includes genes that affect the growth and spread of tumors. Oxygen and nutrients, which are essential to the survival and growth of tumors, are supplied by blood vessels, and the growth of new blood vessels (angiogenesis) is important to tumor progression. Angiogenesis is stimulated by growth factors and other proteins encoded by genes whose expression is carefully regulated in normal cells. In tumor cells, genes encoding these proteins are often overexpressed compared with normal cells, and inhibitors of angiogenesis-promoting factors may be inactivated or underexpressed. For example, vascular endothelial growth factor (VEGF) is a key protein involved in angiogenesis, and it is often overexpressed in cancer cells. This leads to new blood vessels,

allowing the tumor to grow rapidly. At least one inherited cancer—von Hippel–Lindau disease, in which people develop multiple types of tumors—is caused by the mutation of a gene that affects angiogenesis.

In the development of many cancers, the primary tumor gives rise to cells that spread to distant sites, producing secondary tumors. This is called metastasis and is the cause of death in 90% of human cancer deaths. Metastasis is a complex process, requiring that cancer cells escape from the primary tumor site, evade the immune system, travel to a distant site, adhere to existing cells at the new site, and receive nutrients and growth factors necessary for sustained growth. The process requires numerous cellular changes induced by somatic mutation at a number of genes.

By using microarrays to measure levels of gene expression in tumors, researchers have identified genes that are transcribed at a significantly higher rate in metastatic cells than in nonmetastatic cells. For example, one study detected a set of 95 genes that were overexpressed or underexpressed in a population of metastatic breast cancer cells that were strongly metastatic to the lung, compared with a population of cells that were only weakly metastatic to the lung. Genes that contribute to metastasis often encode components of the extracellular matrix and the cytoskeleton. Others encode adhesion proteins, which help hold cells together.

Much recent research has focused on how abnormal cancer cells escape the immune system (see Chapter 22). In 2018, James P. Allison and Tasuku Honjo won the Nobel Prize in physiology or medicine for their research on how the immune system can be stimulated to recognize and attack cancer cells. Research on the role of immunity in cancer continues, and immunotherapy is being used in the treatment of cancer.

Advances in sequencing technology have now made it possible to study how metastasized tumor cells differ from normal cells, as well as from those of the primary tumor from which they were derived. In one experiment, researchers sequenced the entire genome of cells from a metastasized breast cancer tumor and compared it with the genome of normal cells from the same person and with the genome of the primary tumor, which had been removed from the patient nine years earlier. The researchers found 32 different somatic mutations in the coding regions of genes from the metastasized tumor cells, 19 of which were not detected in the primary tumor. This finding suggests that the metastasized tumor underwent considerable genetic changes in its nine-year evolution from the primary tumor. In contrast, another study of a breast cancer metastasis found only two mutations that were not present in the primary tumor, but in this case, the metastasis had evolved in only one year.

A long-standing view has been that metastasized tumors originate from a single cell that breaks off from the primary tumor and travels

to a new site. A recent study that carried out genome sequencing of metastatic prostate tumors has revealed that metastases are sometimes composed of cells from more than one subclone found within the primary tumor. This finding shows that the metastases were seeded by multiple cells from the primary tumor, a process termed polyclonal seeding. There was also evidence that some metastatic tumors received cells from the primary tumor as well as from metastatic tumors at other sites in the body, a process called cross-seeding.

CONCEPTS

Mutations in genes that encode components of DNA-repair systems are often associated with cancer; these mutations increase the rate at which mutations are retained and result in an increased number of mutations in proto-oncogenes, tumor-suppressor genes, and other genes that contribute to cell proliferation. Mutations that allow telomerase to be expressed in somatic cells and those that affect vascularization and metastasis can also contribute to cancer progression.

✔ CONCEPT CHECK 5

Which type of mutation in telomerase is associated with cancer cells?

a. Mutations that produce an inactive form of telomerase
b. Mutations that decrease the expression of telomerase
c. Mutations that increase the expression of telomerase
d. All of the above

MicroRNAs and Cancer

MicroRNAs (miRNAs) are a class of small RNA molecules that pair with complementary sequences on mRNA and degrade the mRNA or inhibit its translation (see Section 14.5). Given the fact that miRNAs are important in controlling gene expression and development, it is not surprising that they are also associated with tumor development. Many tumor cells exhibit widespread reduction in the expression of many miRNAs. Researchers have genetically engineered mouse tumor cells that lacked the machinery to generate miRNAs and found that these cells showed enhanced tumor progression when implanted into mice. Interestingly, this effect was seen only in cells that had already initiated tumor development, suggesting that miRNAs play a role in later stages of tumor progression.

Lowered levels of miRNAs may contribute to cancer by allowing oncogenes that are normally controlled by the miRNAs to be expressed at high levels. For example, let-7 miRNA normally controls the expression of the *ras* oncogene, probably by binding to complementary sequences in the 3′ untranslated region of *ras* mRNA and inhibiting translation. In lung-cancer cells, levels of let-7 miRNA are often low, allowing the Ras protein to be highly expressed, which then leads to the development of lung cancer.

A transcription factor called c-MYC is often expressed at high levels in cancer cells. Evidence suggests that c-MYC helps to drive cell

proliferation and the development of cancer. Among other effects, c-MYC binds to the promoters of miRNA genes and decreases their transcription, decreasing the abundance of the miRNAs. Some of these miRNAs are known to suppress tumor development. Research has shown that if, through genetic manipulation, the miRNAs are expressed at high levels, the development of tumors decreases. All of these findings suggest that altered expression of miRNAs plays an important role in cancer.

In other cases, overexpression of miRNAs has been associated with cancer. For example, several miRNAs have been implicated in the process of metastasis. A particular miRNA called miR-10b has been associated with the formation of metastatic breast tumors. In one experiment, investigators manipulated a line of breast cancer cells so that miR-10b was overexpressed. When the manipulated cells were injected into mice, many of the mice developed metastatic tumors, demonstrating that high levels of this miRNA appear to promote the spread of cancer cells. Further study revealed that in humans, levels of miR-10b are elevated in metastatic tumors, as compared with breast tumors in metastasis-free patients. Research shows that miR-10b regulates the expression of a number of other genes, including some that are known to suppress the spread of tumor cells. Other miRNAs are known to inhibit metastasis.

Cancer Genome Projects

Formed in 2008, the International Cancer Genome Consortium coordinates efforts to determine the genome sequences of tumors. A goal of the consortium is to completely sequence 500 tumors from each of 50 different types of cancer, along with the genomes of normal tissues from the same patients. This effort is producing important results, revealing the numbers and types of mutations that are associated with particular cancers. The hope is that new cancer-causing genes will be identified, which will lead to a better understanding of the nature of cancer and suggest new targets for cancer treatment. Another research project, called The Cancer Genome Atlas (TCGA), began in the United States in 2005. This project seeks to provide a comprehensive genomic analysis of over 100 different types of cancer cells, including sequencing of all exons across the genome as well as characterization of mRNA expression, DNA methylation, copy-number variations, and microRNAs of the tumor cells.

The genomes of a number of tumors have been sequenced, and many more are currently being sequenced. For example, the entire genome of a small-cell lung carcinoma (a type of lung cancer) was sequenced in 2010 and compared with the genome of normal cells from the same person. More than 22,000 base-pair mutations were identified in the tumor, of which 134 were within protein-encoding genes. The tumor also possessed 58 chromosome rearrangements and 334 copy-number variations (see Chapter 20). In another study that was part of TCGA, researchers examined mRNA expression, microRNAs, DNA methylation, and copy-number variations in 489

ovarian adenocarcinomas and sequenced the DNA of exons from 316 of the tumors. Almost all of the tumors contained mutations in *p53*, a tumor-suppressor gene involved in DNA repair and cell cycle control. Mutations in *BRCA1* and *BRCA2*, two tumor-suppressor genes that are also mutated in breast cancer, occurred in 22% of the tumors. Mutations in seven other genes occurred statistically more often than in normal cells. The results suggested several new approaches for drug treatment of ovarian cancer.

Another series of studies sequenced a number of genes in samples of malignant gliomas, an incurable and deadly form of brain cancer. The researchers examined DNA sequences, copy-number variations, DNA methylation, and RNA expression in these tumors. The analyses revealed mutations in several genes that appear to be important in the development of glioma tumors. All of these genomic studies are providing new insight into the genetic basis of cancer.

The mutations found in cancer genomes can be divided into two types: drivers and passengers. **Drivers** are mutations that drive the cancer process: they directly contribute to the development of cancer. Drivers include mutations in oncogenes, tumor-suppressor genes, DNA-repair genes, and the other types of genes discussed in this chapter that contribute to the progression of cancer. **Passengers** are mutations that arise randomly in the process of tumor development and do not contribute to cancer progression. Many passengers are located in introns (regions between genes) and in other DNA that is not transcribed and translated, but they can also

arise within protein-encoding genes. A major challenge is to determine which of the numerous mutations found in tumors are drivers, and actually contribute to the development of cancer, and which are passengers, with no effect.

THINK-PAIR-SHARE Question 4

23.3 Epigenetic Changes Are Often Associated with Cancer

Epigenetic changes—alterations to chromatin structure that affect gene expression (see Chapter 21)—are seen in many cancer cells. Two broad lines of evidence suggest that epigenetic changes play an important role in cancer progression. First, genes encoding proteins that are important regulators of epigenetic changes are often mutated in some types of cancer. For example, almost 90% of cases of follicular lymphoma exhibit mutations in the *MLL2* gene, which encodes a histone methyltransferase; this enzyme adds methyl groups to DNA, a type of epigenetic modification that alters chromatin structure and affects transcription. Similarly, the *UTX* gene, which encodes a histone demethylase (an enzyme that removes methyl groups from histone proteins), is mutated in a number of different types of cancer.

A second line of evidence suggesting that epigenetic alterations are important in cancer comes from recent genomic studies that have compared the chromatin structure of cancer cells with that of normal cells from the same individual. These studies often find that the cancer cells have significant alterations to their DNA methylation patterns and histone structure. One type of epigenetic alteration often observed in cancer cells is an overall lower level of DNA methylation (hypomethylation). As discussed in Chapter 17,

DNA methylation is often associated with repression of transcription. It is assumed that hypomethylation leads to transcription of oncogenes, which then stimulate cancer. Some evidence also suggests that hypomethylation causes chromosome instability, a hallmark of many tumors. Tumor cells from mice that have been genetically engineered to have reduced DNA methylation show increased gains and losses of chromosomes, but how hypomethylation might cause chromosome instability is unclear.

A number of studies have observed that, although the overall level of DNA methylation is often lower in cancer cells, some specific CpG islands (see Chapter 17) have extra methylation (are hypermethylated). For example, one study found that 5% to 10% of normally unmethylated CpG islands located at promoters become abnormally methylated in cancer cells. This excess methylation may inhibit transcription of tumor-suppressor genes, thus stimulating the development of cancer. Methylation of the promoter of the *Apaf-1* gene is seen in many malignant melanoma cells. *Apaf-1* helps bring about apoptosis in cells with damaged DNA; methylation of its promoter reduces the expression of *Apaf-1*, interrupting the process of apoptosis and allowing abnormal cancer cells to survive.

Research has also demonstrated that the histone proteins in nucleosomes, the fundamental units of chromatin, are often abnormally modified in cancer cells. Modification of histone

proteins, including methylation and acetylation, alters chromatin structure and affects whether transcription occurs. Genome-wide patterns of histone acetylation are often altered in cancer cells. Epigenetic processes are receiving increasing attention from cancer researchers because they may be amenable to drug therapy.

CONCEPTS

Epigenetic changes, including DNA methylation and histone modification, are often associated with cancer.

✔ CONCEPT CHECK 6

Hypermethylation is thought to contribute to cancer by

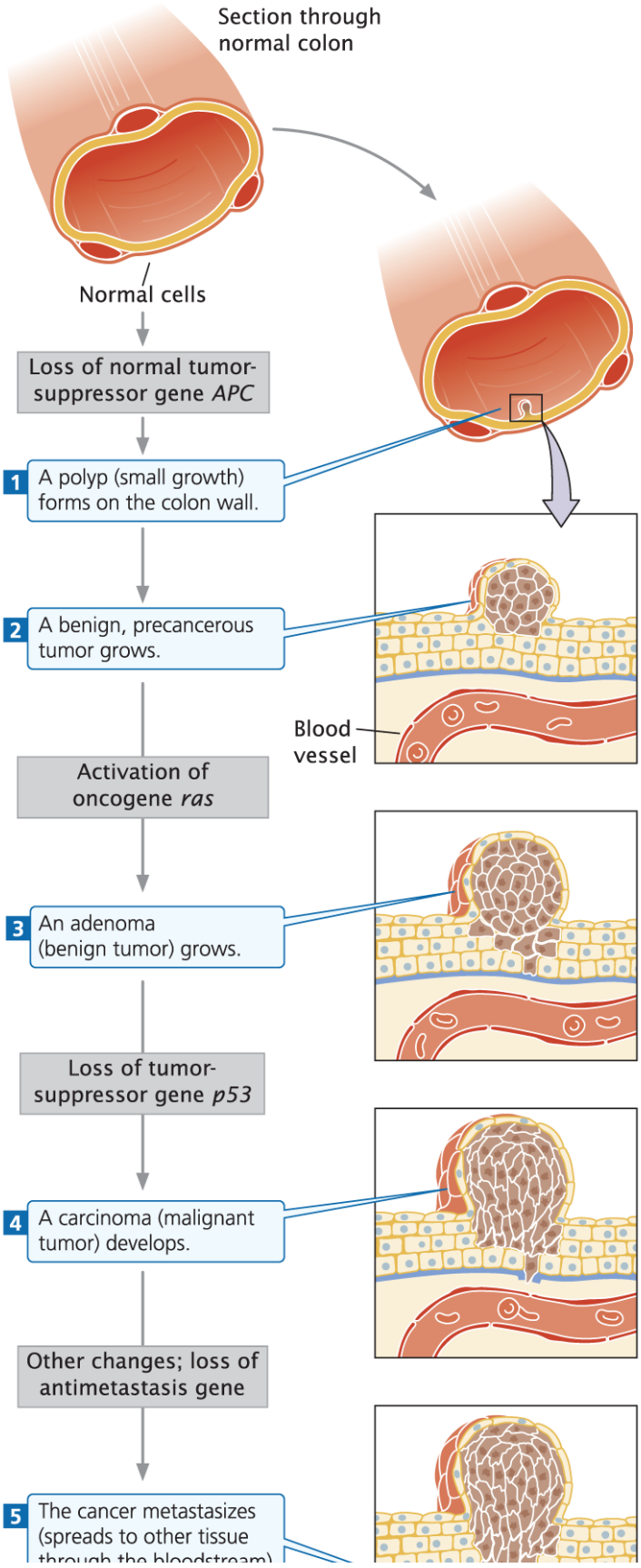
a. inhibiting DNA replication.
b. inhibiting the expression of tumor-suppressor genes.
c. stimulating the translation of oncogenes.
d. stimulating telomerase.

23.4 Colorectal Cancer Arises Through the Sequential Mutation of a Number of Genes

Most cancers arise from mutations in several genes, often a combination of oncogenes and tumor-suppressor genes. Colorectal cancer is an excellent example of how the accumulation of successive genetic defects can lead to cancer.

Colorectal cancer arises in the cells lining the colon and rectum. Almost 146,000 new cases of colorectal cancer are diagnosed each year in the United States, where this cancer is responsible for more than 51,000 deaths annually. If detected early, colorectal cancer can be treated successfully; consequently, there has been much interest in identifying the molecular events responsible for the initial stages of this cancer.

Colorectal cancer is thought to originate as benign tumors called adenomatous polyps (Figure 23.10). Initially, these polyps are microscopic, but in time they enlarge, and their cells acquire the abnormal characteristics of cancer cells. In the later stages of the disease, the tumor may invade the muscle layer surrounding the gut and metastasize. The progression of the disease is slow: it may take 10 to 35 years for a benign tumor to develop into a malignant tumor.


Section through normal colon
Normal cells
Loss of normal tumor-suppressor gene *APC*
1 A polyp (small growth) forms on the colon wall.
2 A benign, precancerous tumor grows.
Blood vessel
Activation of oncogene *ras*
3 An adenoma (benign tumor) grows.
Loss of tumor-suppressor gene *p53*
4 A carcinoma (malignant tumor) develops.
Other changes; loss of antimetastasis gene
5 The cancer metastasizes (spreads to other tissue

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23.10 Mutations in multiple genes contribute to the progression of colorectal cancer.

Description
A flowchart is shown with pictures. A section through normal colon is shown. Normal cells from the colon lead to loss of normal tumorsuppressor gene A P C. A picture shows a lump on the inside wall of the colon. A magnified image is then shown. The image has several cells and at the center is a lump with abnormally shaped cells. A blood vessel is shown below with spherical and bean-shaped cells. Two captions are as follows: “A polyp (small growth) forms on the colon wall.” and “A benign, precancerous tumor grows.” It leads to activation of oncogene ras, which further leads to loss of tumorsuppressor gene p 53. A caption reads, “An adenoma (benign tumor) grows.” The picture shows an even larger lump, which grows even more in the next picture spreading to the normal cells below. Another caption reads, “A carcinoma (malignant tumor) develops.” It then leads to other changes; loss of antimetastasis gene. A picture shows cancer cells breaking the normal cells and entering the blood vessel below. A caption reads, “The cancer metastasizes (spreads to other tissue through the bloodstream).”

Most cases of colorectal cancer are sporadic, developing in people with no family history of the disease, but a few families display a clear genetic predisposition to it. In one form of hereditary colon cancer, known as familial adenomatous polyposis coli, hundreds or thousands of polyps develop in the colon and rectum; if these polyps are not removed, one or more almost invariably become malignant.

Because polyps and tumors of the colon and rectum can be easily observed and removed with a colonoscope (a fiber-optic instrument

used to view the interior of the rectum and colon), much is known about the progression of colorectal cancer, and some of the genes responsible for its clonal evolution have been identified. Mutations in these genes are responsible for the successive steps of colorectal-cancer progression. Among the earliest steps is a mutation that inactivates the *APC* gene; this mutation increases the rate of cell division, leading to polyp formation (see Figure 23.10). A person with familial adenomatous polyposis coli inherits one defective copy of the *APC* gene, and defects in this gene are associated with the numerous polyps that appear in those who have this disorder. Mutations in *APC* are also found in polyps that develop in people who do not have familial adenomatous polyposis coli.

Mutations of the *ras* oncogene usually occur later, in larger polyps consisting of cells that have acquired some genetic mutations. As discussed earlier in this chapter, the normal *ras* proto-oncogene is a key player in a signal-transduction pathway that relays a signal from growth factors to the nucleus, where the signal stimulates cell division. When *ras* is mutated, the protein it produces continually relays a stimulatory signal for cell division, even when growth factors are absent.

Mutations in tumor-suppressor gene *p53* and other genes appear still later in tumor progression; these mutations are rare in polyps but common in malignant cells. Many colorectal cancers have mutations in *p53*. Because *p53* prevents the replication of cells with genetic

damage and affects proper chromosome segregation, mutations in *p53* can allow a cell to rapidly acquire further gene and chromosome mutations, which then contribute to further proliferation and invasion of surrounding tissues.

Recent research also shows that inflammation plays a role in the development of colorectal cancer. For example, people with inflammatory bowel disease are at increased risk for developing colorectal cancer. (Inflammatory bowel disease is chronic inflammation of the digestive tract, often accompanied by severe diarrhea, pain, fatigue, and weight loss.) Although the precise mechanism by which inflammation contributes to cancer is unknown, immune-system cells and the proteins they secrete (see Section 22.6) are likely to affect many of the steps in the progression of cancer.

The sequence of steps just outlined is not the only route to colorectal cancer, and the mutations need not occur in the order presented here. However, this sequence is a common pathway by which colon and rectal cells become cancerous.

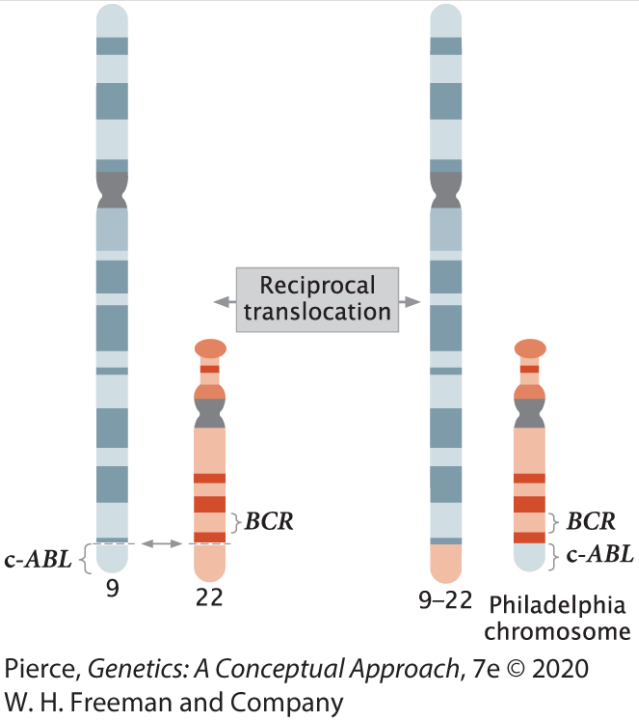
23.5 Changes in Chromosome Number and Structure Are Often Associated with Cancer

Most tumors contain cells with chromosome mutations. For many years, geneticists argued about whether these chromosome mutations were the cause or the result of cancer. Some types of tumors are consistently associated with *specific* chromosome mutations; for example, most cases of chronic myelogenous leukemia are associated with a specific reciprocal translocation between chromosomes 22 and 9. These types of associations suggest that chromosome mutations contribute to the cause of the cancer. Yet many cancers are not associated with specific types of chromosome abnormalities, and individual *gene* mutations are now known to contribute to many types of cancer. Nevertheless, as we have noted, chromosome instability is a general feature of cancer cells, causing them to accumulate chromosome mutations, which then affect individual genes that may contribute to the cancer process. Thus, chromosome mutations appear to both *cause* cancer and *result* from it.

At least three types of chromosome rearrangements—deletions, inversions, and translocations—are associated with certain types of cancer. Deletions can result in the loss of one or more tumor-suppressor genes. Inversions and translocations contribute to

cancer in several ways. First, the chromosome breaks that accompany these mutations can lie within tumor-suppressor genes, disrupting their function and leading to cell proliferation.

Second, translocations and inversions can bring together sequences from two different genes, generating a fusion protein that stimulates some aspect of the cancer process. Fusion proteins are seen in most cases of chronic myelogenous leukemia, which affects bone-marrow cells. Most people with chronic myelogenous leukemia have a reciprocal translocation between the long arm of chromosome 22 and the tip of the long arm of chromosome 9 (**Figure 23.11**). This translocation produces a shortened chromosome 22, called the Philadelphia chromosome because it was first discovered in Philadelphia. At the end of a normal chromosome 9 is a potential cancer-causing gene called c-*ABL*. As a result of the translocation, part of the c-*ABL* gene is fused with the *BCR* gene from chromosome 22. The protein produced by this *BCR*–c-*ABL* fusion gene is much more active than the protein produced by the normal c-*ABL* gene; the fusion protein stimulates increased, unregulated cell division and eventually leads to leukemia.



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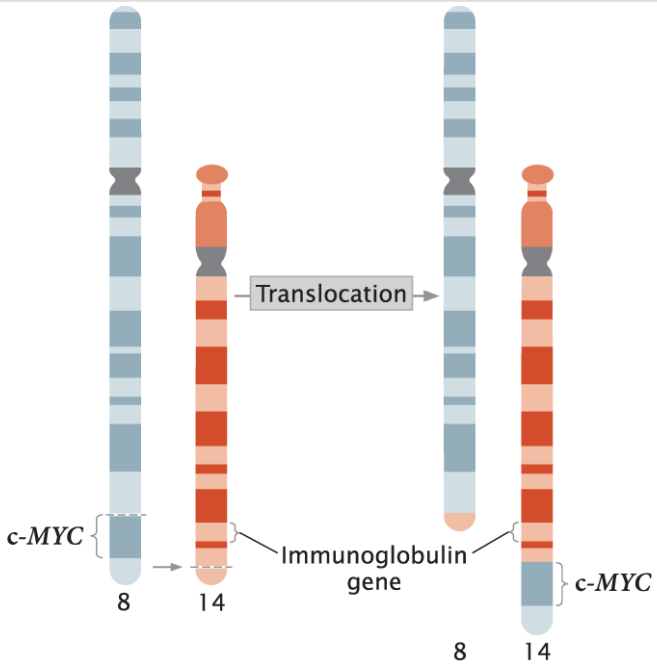
23.11 A reciprocal translocation between chromosomes 9 and 22 causes chronic myelogenous leukemia.

Description
The illustration shows a long linear chromosome 9 labeled for c-A B L gene and short linear chromosome 22 labeled for B C R gene. Both chromosomes have bands of different colors. Reciprocal translocation occurs and both the genes are now on the short chromosome labeled the Philadelphia chromosome, while the long chromosome is labeled 9-22.

A third mechanism by which chromosome rearrangements can produce cancer is the transfer of a potential cancer-causing gene to a new location, where it is activated by different regulatory sequences. Burkitt lymphoma is a cancer of the B cells, the lymphocytes that produce antibodies (see Section 22.6). Many people with Burkitt lymphoma possess a reciprocal translocation between chromosome

8 and chromosome 2, 14, or 22 (Figure 23.12). This translocation relocates a gene called c-*MYC* from the tip of chromosome 8 to a position on chromosome 2, 14, or 22 that is next to a gene that encodes an immunoglobulin protein. At this new location, c-*MYC*, a cancer-causing gene, comes under the control of regulatory sequences that normally activate the production of immunoglobulins, and c-*MYC* is therefore expressed in B cells. The c-MYC protein stimulates the division of the B cells and leads to Burkitt lymphoma.



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23.12 A reciprocal translocation between chromosomes 8 and 14 causes Burkitt lymphoma.

Description

The illustration shows a long linear chromosome 8 labeled for c-M Y C gene and shorter linear chromosome 14 labeled for immunoglobulin gene. Both chromosomes have bands

of different colors. Translocation occurs and both the genes are now on the short chromosome 14.

CONCEPTS

Many tumors contain a variety of chromosome mutations. Some types of tumors are associated with specific deletions, inversions, and translocations. Deletions can eliminate or inactivate genes that control the cell cycle; inversions and translocations can cause breaks in genes that suppress tumors, fuse genes to produce cancer-causing proteins, or move genes to new locations, where they are under the influence of different regulatory sequences.

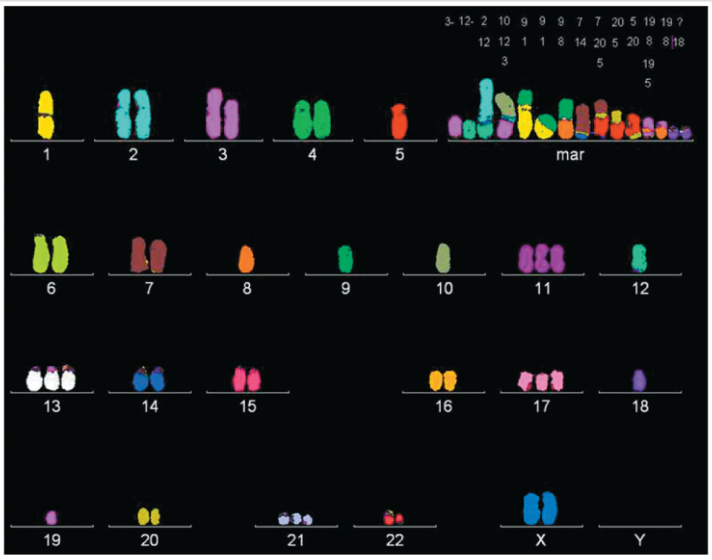
✔ CONCEPT CHECK 7

Chronic myelogenous leukemia is usually associated with which type of chromosome rearrangement?

a. Duplication
b. Deletion
c. Inversion
d. Translocation

Most advanced tumors contain cells that exhibit a dramatic variety of chromosome anomalies, including extra chromosomes, missing chromosomes, and chromosome rearrangements (Figure 23.13). Some cancer researchers believe that cancer is initiated when genetic changes take place that cause the genome to become unstable, generating numerous chromosome abnormalities that then alter the expression of oncogenes and tumor-suppressor genes. A number of genes that contribute to genomic instability and lead to

missing or extra chromosomes (aneuploidy) have now been identified. ❯ **TRY** [PROBLEM 31]



Courtesy Dr. Peter Duesberg, UC Berkeley.

23.13 Cancer cells often possess chromosome abnormalities, including extra chromosomes, missing chromosomes, and chromosome rearrangements. Shown here are chromosomes from a colon-cancer cell, which has numerous chromosome abnormalities. For comparison, see the normal karyotype in Figure 2.6a.

Description

Chromosome 1 shows a single chromosome. Chromosome 2 shows a pair of chromosomes. Chromosome 3 shows a pair of chromosomes, one of which is shorter than the other. Chromosome 4 shows two short chromosomes of equal length. Chromosome 5 shows a single short chromosome. Chromosomes labeled mar shows fourteen chromosomes, eight of which are shorter, one is spherical, one is curvy, and rest are longer. Chromosome 6 and 7 show a pair of two curvy short chromosomes. Chromosomes 8, 9, and 10 shows single short chromosomes. Chromosome 11 shows three short chromosomes of equal length. Chromosome 12 shows a single short chromosome. Chromosome 13 shows three short chromosomes of equal length. Chromosome 14, 15, and 16 show a pair of two short chromosomes. Chromosome 17 shows three short chromosomes. Chromosome 18 shows a single short chromosome.

Chromosome 19 shows an even shorter chromosome. Chromosome 20 shows a pair of short chromosomes. Chromosome 21 shows three very short chromosomes. Chromosome 22 shows a pair of very short chromosomes. X chromosome shows a pair of large chromosomes and the space for Y chromosome is empty.

23.6 Viruses Are Associated with Some Cancers

As mentioned earlier in this chapter, viruses are responsible for a number of cancers in animals, and there is evidence that viruses contribute to at least a few cancers in humans (Table 23.6). For example, about 95% of all women with cervical cancer are infected with **human papillomavirus (HPV)**. Similarly, infection with the virus that causes hepatitis B increases the risk of liver cancer in some people. The Epstein–Barr virus, which is responsible for mononucleosis, has been linked to several types of cancer that are prevalent in parts of Africa, including Burkitt lymphoma.

TABLE 23.6 Some human cancers associated with viruses

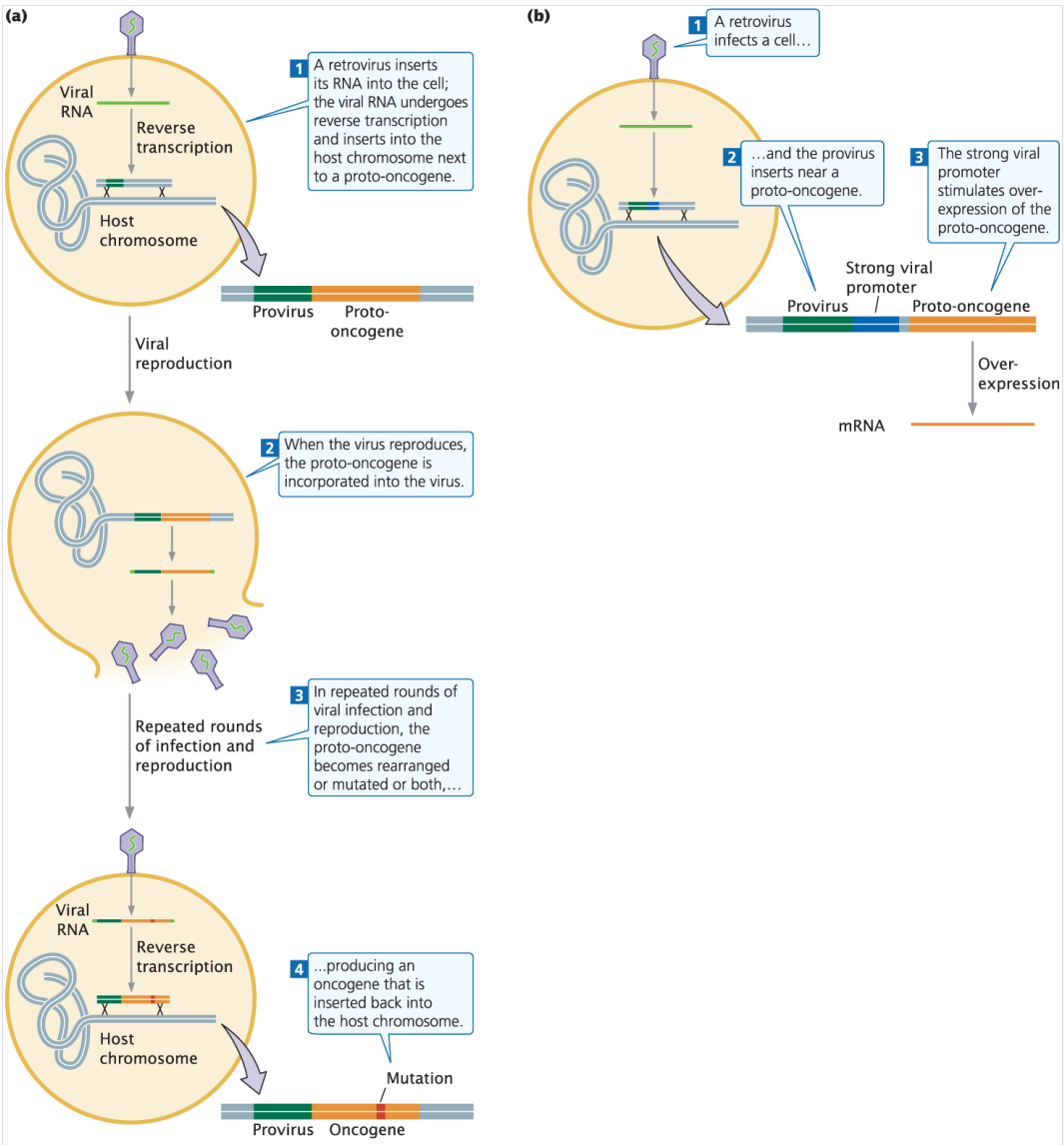
Virus	Cancer
Human papillomavirus (HPV)	Cervical, penile, and vulvar cancers
Hepatitis B virus	Liver cancer
Human T-cell leukemia virus 1 (HTLV-1)	Adult T-cell leukemia
Human T-cell leukemia virus 2	Hairy-cell leukemia

(HTLV-2)	
Epstein–Barr virus	Burkitt lymphoma, nasopharyngeal cancer, Hodgkin lymphoma
Human herpesvirus	Kaposi sarcoma
Merkel cell polyomavirus	Merkel cell carcinoma

Note: Some of these associations between cancer and viruses exist only in certain populations and geographic areas.

Retroviruses and Cancer

Many of the viruses that cause cancer in animals are retroviruses. In Section 23.2, we saw how studies of the Rous sarcoma retrovirus in chickens led to the identification of oncogenes in humans. Retroviruses sometimes cause cancer by mutating or rearranging host genes, converting proto-oncogenes into oncogenes (**Figure 23.14a**). Another way in which viruses can contribute to cancer is by altering the expression of host genes (**Figure 23.14b**). Retroviruses often contain strong promoters to ensure that their own genetic material is transcribed by the host cell. If the provirus inserts near a proto-oncogene, viral promoters can stimulate high levels of expression of the proto-oncogene, leading to cell proliferation.



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23.14 Retroviruses can cause cancer by (a) mutating or rearranging proto-oncogenes or (b) inserting strong promoters near proto-oncogenes.

Description

Part “a” shows a cell being infected with a retrovirus. The cell shows viral R N A inside which forms viral D N A through reverse transcription and incorporates into the host chromosome. The host chromosome shows two parts provirus and proto-oncogene. A caption reads, “A retrovirus inserts its R N A into the cell; the viral R N A undergoes reverse transcription and inserts into the host chromosome next to a proto-oncogene.” Viral reproduction occurs then and new viruses exit the host cell after its lysis. A caption

reads, “When the virus reproduces, the proto-oncogene is incorporated into the virus.” After repeated rounds of infection and reproduction, another virus infects a host cell. The host chromosome from this cell has provirus and oncogene with mutation. A caption reads, “In repeated rounds of viral infection and reproduction, the proto-oncogene becomes rearranged or mutated or both, producing an oncogene that is inserted back into the host chromosome.” Part “b” again shows a cell being infected with a retrovirus. The cell shows viral R N A inside which forms viral D N A through reverse transcription and incorporates into the host chromosome. A caption reads, “A retrovirus infects a cell and the provirus inserts near a proto-oncogene.” This time the host chromosome has a provirus, strong-viral promoter, and proto-oncogene. Over-expression of proto-oncogene produces an m R N A. A caption reads, “The strong viral promoter stimulates overexpression of the proto-oncogene.”

There are only a few retroviruses that cause cancer in humans. HTLV-1, the first human retrovirus discovered, is associated with human adult T-cell leukemia. Other human cancers are associated with DNA viruses, which, like retroviruses, integrate into the host chromosome but, unlike retroviruses, do not use reverse transcription.

Human Papillomavirus and Cervical Cancer

Human papillomavirus (HPV) is a DNA virus that causes warts and other types of benign tumors of epithelial cells. More than 100 different types of HPV are known, about 30 of which are sexually transmitted and cause genital warts. A few of them are associated with cervical cancer. In the United States, 70% of the cases of

cervical cancer are caused by HPV-16 and HPV-18. One way that HPVs cause cancer is by producing proteins that attach to and inactivate the protein products of *RB* and *p53*. As we have seen, the proteins encoded by these two genes play key roles in the regulation of the cell cycle. When these proteins are inactivated, cells are stimulated to progress through the cell cycle and divide without the normal controls that prevent cell proliferation. Human papillomaviruses can also stimulate cancer by causing high expression of growth-promoting proteins.

Most sexually active women and men in the United States are infected with HPV, but only a small number of women with HPV will ever develop cervical cancer. The risk of infection by HPV can be reduced by limiting the number of sexual partners and by a vaccine that was approved by the U.S. Food and Drug Administration for women in 2006 and for men in 2009. This vaccine is highly effective against four of the most common HPVs associated with cervical cancer. The U.S. Centers for Disease Control recommends that all boys and girls aged 11 or 12 years be vaccinated. A national survey conducted in the United States in 2014 reported that 60% of girls aged 13–17 years had received at least one dose of the vaccine, but only 42% of boys aged 13–17 years had received at least one dose. About 40% of girls and 22% of boys received all three of the recommended doses of the HPV vaccine.

In spite of the large number of women infected with HPV, the incidence of cervical cancer in the United States has declined 75% in

the past 40 years. The primary reason for this decline is widespread use of the Pap test, which detects early stages of cervical cancer as well as cervical dysplasia, a precancerous growth that can be removed before it develops into cancer.

Although rare in the United States, cervical cancer is the second-most common cause of cancer in women worldwide, with high incidences in many developing countries, such as those of Sub-Saharan Africa, South Asia, and South and Central America. An estimated 510,000 women throughout the world develop cervical cancer each year, and 288,000 die from the disease. The primary reason for the high incidence and death rate in developing regions is lack of access to cervical-cancer screening procedures, such as the Pap test. Making the HPV vaccine against cervical cancer available in these regions could greatly reduce the incidence of cervical cancer.

THINK-PAIR-SHARE Question 5

CONCEPTS

Viruses contribute to a few cancers in humans by mutating or rearranging host genes, which then contribute to cell proliferation, or by altering the expression of host genes.

Chapter 23 Review

CHAPTER SUMMARY

Section 23.1 Learning Objective: Be able to explain how cancer is a genetic disease, in spite of the fact that most cancers are not inherited.

- Cancer is fundamentally a genetic disease, arising from somatic mutations in multiple genes that affect cell division and proliferation. If one or more mutations are inherited, then fewer additional mutations are required for cancer to develop.
- A mutation that allows a cell to divide rapidly provides the cell with a growth advantage; that cell gives rise to a clone of cells having the same mutation. Within this clone, other mutations may occur that provide additional growth advantages; cells with these additional mutations become dominant in the clone. In this way, the clone evolves.
- Environmental factors play an important role in the development of many cancers by increasing the rate of somatic mutations.

Section 23.2 Learning Objective: Be able to summarize the different types of genes that contribute to cancer.

- Oncogenes are dominant-acting mutated copies of normal genes (proto-oncogenes) that stimulate cell division. Tumor-suppressor genes normally inhibit cell division; recessive mutations in these genes may contribute to cancer. Sometimes the mutation of a single copy of a tumor-suppressor gene is sufficient to cause cancer, a phenomenon known as haploinsufficiency.
- The cell cycle is controlled by cyclins and cyclin-dependent kinases. Mutations in genes that control progression through the cell cycle are often associated with cancer.
- Signal-transduction pathways conduct external signals that bring about intracellular responses. These pathways consist of a series of proteins that are activated and inactivated in a cascade of reactions. Mutations in the components of signal-transduction pathways can disrupt the cell cycle and may contribute to cancer.
- Defects in DNA-repair genes often increase the overall mutation rate of other genes, leading to defects in proto-oncogenes and tumor-suppressor genes, which can contribute to cancer progression.
- Mutations in sequences that regulate telomerase allow cells to divide indefinitely, contributing to cancer progression.
- Tumor progression is also affected by mutations in genes that promote vascularization and the spread of tumors.
- Many tumor cells exhibit a widespread reduction in the expression of many miRNAs, suggesting that a reduction in miRNA control of gene expression may play a role in tumor progression.

Section 23.3 Learning Objective: Be able to explain how epigenetic changes contribute to cancer.

- Epigenetic changes in chromatin structure, including DNA methylation and histone modification, are often associated with cancer.

Section 23.4 Learning Objective: Be able to outline the sequential mutations in colon cells that lead to colorectal cancer.

- Colorectal cancer offers a model system for understanding tumor progression in humans. Initial mutations stimulate cell division, leading to a small benign polyp. Additional mutations allow the polyp to enlarge, invade the muscle layer of the gut, and eventually spread to other sites. Mutations in particular genes affect different stages of this progression.

Section 23.5 Learning Objective: Be able to describe different types of changes in chromosome number and structure that contribute to some types of cancer.

- Some cancers are associated with specific chromosome mutations, including chromosome deletions, inversions, and translocations. Deletions may cause cancer by removing or disrupting genes that suppress tumors; inversions and translocations may break tumor-suppressing genes, or they may move genes to positions next to different regulatory sequences, which alter their expression.

- Mutations in some genes cause or allow chromosomes to segregate improperly, leading to aneuploidy that can contribute to cancer.

Section 23.6 Learning Objective: Be able to explain how viruses can cause cancer.

- Viruses are associated with some cancers; they contribute to cell proliferation by mutating or rearranging host genes or by altering the expression of host genes.

WORKED PROBLEM

In some cancer cells, a specific gene has been duplicated many times. Is this gene likely to be an oncogene or a tumor-suppressor gene? Explain your reasoning.

» Solution Strategy

What information is required in your answer to the problem?

Whether the duplicated gene is likely to be an oncogene or a tumor-suppressor gene, and why.

What information is provided to solve the problem?

In cancer cells, the gene has been duplicated many times.

For help with this problem, review:

Oncogenes and Tumor-Suppressor Genes in Section 23.2.

» Solution Steps

The gene is likely to be an oncogene. Oncogenes stimulate cell proliferation and act in a dominant manner. Therefore, extra copies of an oncogene will result in cell proliferation and cancer. Tumor-suppressor genes, on the other hand, suppress cell proliferation and act in a recessive manner; a single copy of a tumor-suppressor gene is sufficient to prevent cell proliferation. Therefore, extra copies of the tumor-suppressor gene will not lead to cancer.

Recall: An oncogene is an accelerator of cell division, while a tumor-suppressor gene is a brake.

IMPORTANT TERMS

malignant
metastasis
clonal evolution
oncogene
tumor-suppressor gene
proto-oncogene
loss of heterozygosity

haploinsufficiency
cyclin-dependent kinase (CDK)
cyclin
signal-transduction pathway
driver
passenger
human papillomavirus (HPV)

ANSWERS TO CONCEPT CHECKS

1. Retinoblastoma results from at least two separate genetic defects, both of which are necessary for cancer to develop. In sporadic cases, two successive mutations must occur in a single cell, which is unlikely, and therefore, the cancer typically affects only one eye. In people who have inherited one of the two required mutations, every cell contains this mutation, and so a single additional mutation is all that is required for cancer to develop. Given the millions of cells in each eye, there is a high probability that the second mutation will occur in at least one cell of each eye, producing tumors in both eyes and the inheritance of this type of retinoblastoma.
2. Oncogenes have a stimulatory effect on cell proliferation. Mutations in oncogenes are usually dominant, because a mutation in a single copy of the gene is usually sufficient to produce a stimulatory effect. Tumor-suppressor genes inhibit cell proliferation. Mutations in tumor-suppressor genes are

generally recessive, because both copies must be mutated to remove all inhibition.

3. d
4. a
5. c
6. b
7. d

Chapter 23 Assessment

COMPREHENSION QUESTIONS

Section 23.1

1. What types of evidence indicate that cancer arises from genetic changes?

2. How is cancer different from most other types of genetic diseases?

3. Outline Knudson's two-hit hypothesis of retinoblastoma, and describe how it helps to explain unilateral and bilateral cases of retinoblastoma.

4. Briefly explain how cancer progresses through clonal evolution.

Section 23.2

5. What is the difference between an oncogene and a tumor-suppressor gene? Give some examples of the functions of proto-oncogenes and tumor-suppressor genes in normal cells.

6. What is haploinsufficiency? How might it affect cancer risk?

7. How do cyclins and CDKs differ? How do they interact in controlling the cell cycle?

8. Briefly outline the events that control the progression of cells through the G_1/S checkpoint in the cell cycle.

9. Briefly outline the events that control the progression of cells through the G_2/M checkpoint in the cell cycle.

10. What is a signal-transduction pathway? Why are mutations in components of signal-transduction pathways often associated with cancer?

11. How is the Ras protein activated and inactivated?

12. Why do mutations in genes that encode DNA-repair enzymes often produce a predisposition to cancer?

13. What role do telomeres and telomerase play in cancer progression?

Section 23.3

14. How is an epigenetic change different from a mutation?

15. How is DNA methylation related to cancer?

Section 23.4

16. Briefly outline some of the genetic changes commonly associated with the progression of colorectal cancer.

Section 23.5

17. *Explain how chromosome deletions, inversions, and translocations can cause cancer.

18. Briefly outline how the Philadelphia chromosome leads to chronic myelogenous leukemia.

19. What is genomic instability? Give some ways in which genomic instability may arise.

Section 23.6

20. *How do viruses contribute to cancer?

APPLICATION QUESTIONS AND PROBLEMS

Introduction

21. CONNECT What characteristics of the pedigrees shown in **Figure 23.1** suggest that Li-Fraumeni syndrome is inherited as an autosomal dominant trait? (See Chapter 6 for a review of pedigrees.)

Section 23.1

22. If cancer is fundamentally a genetic disease, how might an environmental factor such as smoking cause cancer?

23. *Both genes and environmental factors contribute to cancer. Prostate cancer is 39 times more common among people from Utah than among people from Shanghai (see **Table 23.2**). Briefly outline how you might determine whether these differences in the incidence of prostate cancer are due to differences in the genetic makeup of the two populations or to differences in their environments.

Section 23.2

24. *The *TP53* gene, which plays a role in Li-Fraumeni syndrome and in many types of cancer (see the introduction to this chapter), is said to be a tumor-suppressor gene. Which of its characteristics suggest that it is a tumor-suppressor gene, rather than an oncogene?

25. Mutations in the *RB* gene are often associated with cancer. Explain how a mutation that results in a nonfunctional RB protein contributes to cancer.

26. Cells in a tumor contain mutated copies of a particular gene that promotes tumor growth. Gene therapy (see Chapter 19) can be used to introduce a normal copy of this gene into the tumor cells. Would you expect this therapy to be effective if the mutated gene were an oncogene? A tumor-suppressor gene? Explain your reasoning.

27. What would be the effect on the cell cycle of a drug that inhibited each of the following?
    a. MPF
    b. Cyclin-E–CDK
    c. Cyclin-D–CDK

28. What would be the effect of a drug that inhibited the breakdown of cyclin B?

Section 23.3

29.

David Seligson and his colleagues examined epigenetic modifications (see Chapter 21) associated with prostate cancer. They measured levels of histone protein modification in prostate tumors and their association with clinical outcomes (D. B. Seligson et al. 2005. *Nature* 435:1262–1266). They used antibodies to stain for acetylation at three different sites, and for methylation at two different sites, on histone proteins. They found that the degree of histone acetylation and methylation helped predict whether prostate cancer would return within 10 years in the patients who had a prostate tumor removed. Explain how acetylation and methylation might be associated with tumor recurrence in prostate cancer. (Hint: See Chapter 17.)

30. CONNECT Some cancers have been treated with drugs that demethylate DNA (see Section 17.2). Explain how these drugs might work. Do you think the cancer-causing genes that responded to the demethylation are likely to have been oncogenes or tumor-suppressor genes? Explain your reasoning.

Section 23.5

31. *Some cancers are consistently associated with the deletion of a particular part of a chromosome. Does the deleted region contain an oncogene or a tumor-suppressor gene? Explain.

Section 23.6

32. Assume that the provirus in **Figure 23.14** inserts just upstream of a tumor-suppressor gene. Would this insertion be likely to cause cancer? Why or why not?

CHALLENGE QUESTIONS

Section 23.2

33. Many cancer cells are immortal (will divide indefinitely) because they have mutations that allow telomerase to be

expressed. How might this knowledge be used to design anticancer drugs?

34. CONNECT Bloom syndrome is an autosomal recessive disease that exhibits haploinsufficiency. A recent survey showed that people heterozygous for mutations at the *BLM* locus are at increased risk of colon cancer. Suppose you are a genetic counselor (see Chapter 6). A young woman is referred to you whose mother has Bloom syndrome; the young woman's father has no family history of Bloom syndrome. The young woman asks whether she is likely to experience any other health problems associated with her family history of Bloom syndrome. What advice would you give her?

35. Imagine that you discover a large family in which bladder cancer is inherited as an autosomal dominant trait. Briefly outline a series of studies that you might conduct to identify the gene that causes bladder cancer in this family.

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 23.1

1. The risk of dying from various cancers often differs between males and females. For example, the risk of a male dying from bladder cancer is 1 in 106, but the risk for a female is only 1 in 294. Why are rates of death from cancer different in men and women? Provide some possible explanations.

2. A couple has one child with bilateral retinoblastoma. The mother is free from cancer, but the father has unilateral retinoblastoma, and he has a brother who has bilateral retinoblastoma.
   a. If the couple has another child, what is the probability that this next child will have retinoblastoma?
   b. If the next child has retinoblastoma, is it likely to be bilateral or unilateral?
   c. Explain why the father's case of retinoblastoma is unilateral, whereas his child's and brother's cases are bilateral.

3. **Table 23.3** lists occupation as an environmental factor associated with cancer. What occupations do you think might be associated with higher rates of cancer? What about these occupations might create higher cancer rates?

Section 23.2

4. Drivers are mutations that drive the cancer process; passengers are mutations that arise randomly in the process of tumor development and do not contribute to the progression of cancer. How might researchers go about distinguishing

between drivers and passengers? Propose some different approaches.

Section 23.6

5. In 2007, then Texas governor Rick Perry became the first governor in the United States to mandate by executive order the vaccination of all Texas girls aged 11–12 for HPV, arguing that the vaccine prevented cancer. Some conservatives criticized Perry, arguing that the vaccine would encourage sexual promiscuity among children and young adults. The Texas legislature overturned the executive order, and Perry later reversed his position, saying the order was a mistake. Do you think states should require boys and girls to be vaccinated for HPV? Discuss reasons for and against such a requirement.

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Create a concept map that outlines the role of the *TP53* gene and p53 protein in Li-Fraumeni syndrome. Include at least the following terms and phrases in your map: Li-Fraumeni syndrome, predisposition to cancer, autosomal dominant trait,

TP53 gene, p53 protein, tumor-suppressor gene, cell cycle, genome stability, apoptosis, retrotransposition.

Section 23.1

2. Summarize the process by which normal cells become cancerous by creating a concept map that links the following terms: normal cells, clonal evolution, tumor, malignant tumor, metastasis.

Section 23.2

3. Draw a concept map that summarizes the differences between oncogenes and tumor-suppressor genes. Include at least 9 concepts in your map.

Section 23.4

4. With a concept map, summarize the stages in the progression of a normal colon cell to metastatic cancer, including some of the important genes involved in this process. You should include at least 10 concepts or terms in your map.

Section 23.5

5. Using a concept map, outline the different ways that change in chromosome number or structure can lead to cancer. Include the following terms or phrases in your map: chromosome changes, change in chromosome structure, aneuploidy,

genomic instability, genes involved in cell cycle control, deletions, inversions, translocations, loss of tumor-suppressor genes, chromosome breaks, fusion proteins, disruption of tumor-suppressor genes, stimulation of oncogenes.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 24

Quantitative Genetics

Jim Craigmyle/Getty Images.

Methods of quantitative genetics coupled with molecular techniques have been used to identify a gene that determines oil content in corn.

Corn Oil and Quantitative Genetics

In 2019, the world's population was 7.7 billion. The United Nations projects that by 2050, it will increase by another 2.1 billion. Feeding those billions of additional people will be a major challenge for agriculture in the next few decades. Crop plants will have to provide

most of the calories and nutrients required for the world's future population. Furthermore, because of dwindling petroleum supplies and concerns about global warming, plants are increasingly being used as sources of biofuel, placing additional demands on crop production.

To help meet the need for increased crop yields, plant breeders are using the latest genetic techniques in their quest to develop higher-yielding, more efficient crop plants. The power of this approach is demonstrated by research aimed at increasing the oil content of corn. The oil content of corn is an inherited characteristic, but its inheritance is more complex than that of the characteristics we have studied so far; it is not a simple single-gene characteristic, like seed shape in peas. Numerous genes and environmental factors contribute to the oil content of corn. For characteristics such as oil content, several loci frequently interact, and their expression is affected by environmental factors.

Can the inheritance of a complex characteristic such as oil content be studied? Is it possible to predict the oil content of a plant on the basis of its breeding? The answer to both questions is yes—at least in part—but these questions cannot be addressed with the methods we have used for simple genetic characteristics. Instead, we must use statistical procedures that have been developed for analyzing complex characteristics. The genetic analysis of complex characteristics, such as the oil content of corn, is known as **quantitative genetics**.

In 2008, geneticists used a combination of quantitative genetic and molecular techniques to identify a gene that plays a key role in controlling the oil content of corn. First, they conducted crosses between high-oil and low-oil corn plants to identify chromosomal regions that play a role in determining oil production. Chromosomal regions containing genes that influence a quantitative trait are termed quantitative trait loci (QTLs). Through these crosses, the geneticists located several QTLs that affected oil content; one of them was on corn chromosome 6.

Fine-scale gene mapping further narrowed the QTL down to a small region of 4.2 map units (centiMorgans) on chromosome 6. Researchers sequenced DNA from the region and found that it contained five genes, one of which was *DGAT1-2*, a gene known to encode an enzyme that catalyzes the final step in a pathway for triacylglycerol biosynthesis. DNA from the *DGAT1-2* gene in a high-oil-producing strain of corn contained an insertion of a codon that added phenylalanine to the enzyme—an insertion that was missing from a low-oil-producing strain. The researchers confirmed the effect of the additional phenylalanine codon on oil production by producing transgenic corn that contained the extra codon. These transgenic strains produced more oil than transgenic strains without the additional codon. Interestingly, the additional phenylalanine codon is present in wild relatives of corn, suggesting that the codon was lost in the process of domestication or subsequent breeding of modern varieties.

This research suggests that the oil content of corn and other plants might be increased by genetically modifying their *DGAT1-2* genes to contain the additional codon for phenylalanine. Other studies that similarly combine quantitative and molecular analyses have led to the identification of genes that increase the vitamin A content of rice and increase sugar production in tomatoes.

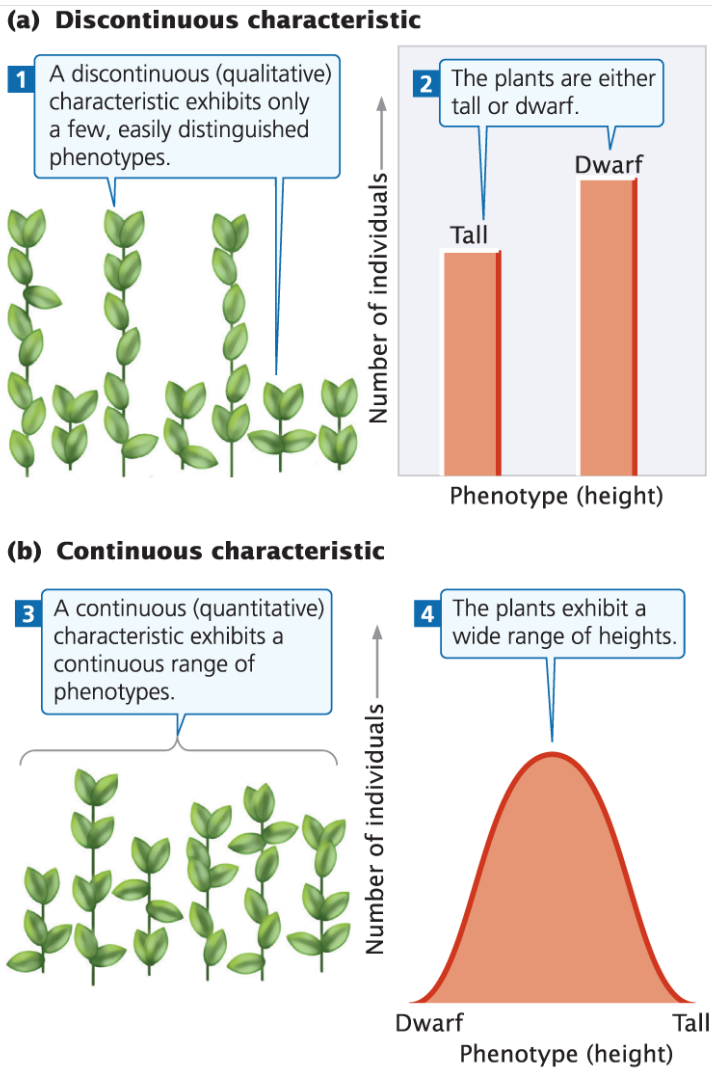
THINK-PAIR-SHARE

- What are some other problems associated with the increasing human population, and how is genetics being used to address them?
- Researchers produced transgenic corn that contained an extra codon for phenylalanine and had a high oil content. Suppose an existing commercial variety of corn was modified in the same way and sold to farmers. Would you consider this variety to be a genetically modified organism (GMO; see Chapter 19)? Should the corn produced by this variety be labeled as a genetically modified food product?

This chapter is about the genetic analysis of complex characteristics, such as the oil content of corn. We begin by considering the differences between quantitative and qualitative genetic characteristics and why the expression of some characteristics varies continuously. We'll see that quantitative characteristics are often influenced by many genes, each of which has a small effect on the phenotype. Next, we'll examine statistical procedures for describing and analyzing quantitative characteristics. We'll consider how much of phenotypic variation can be attributed to genetic and environmental influences. Finally, we'll look at the effects of selection on quantitative characteristics.

24.1 Quantitative Characteristics Are Influenced by Alleles at Multiple Loci

Qualitative, or discontinuous, characteristics possess only a few distinct phenotypes (**Figure 24.1a**). These characteristics are the types studied by Mendel (e.g., round and wrinkled peas) and have been the focus of our attention thus far. Many characteristics, however, vary continuously along a scale of measurement, exhibiting many overlapping phenotypes (**Figure 24.1b**). They are referred to as *continuous characteristics*, and they are also called *quantitative characteristics* because any individual's phenotype must be described by a quantitative measurement. Examples of quantitative characteristics include height, weight, and blood pressure in humans; growth rate in mice; seed weight in plants; and milk production in cattle.



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24.1 Discontinuous and continuous characteristics differ in the number of phenotypes exhibited.

Description

Part a labeled discontinuous characteristic shows tall and dwarf plants arranged randomly. A caption reads, A discontinuous (qualitative) characteristic exhibits only a few, easily distinguished phenotypes. A bar graph on the right shows number of individuals plotted against phenotype (height). The horizontal axis represents Phenotype (height): Tall and dwarf. The vertical axis represents number of individuals. The bar representing tall is shorter than the bar representing dwarf. A caption reads, The plants are either tall or dwarf.

Part b labeled continuous characteristic shows plants of varying heights arranged randomly. A caption reads, A continuous (quantitative) characteristic exhibits a continuous range of phenotypes. A non-linear graph on the right shows number of individuals plotted against phenotype (height). The horizontal axis represents Phenotype (height): Dwarf and tall. The vertical axis represents number of individuals. A bell curve begins from dwarf, near the origin, and ends at tall. A caption reads, The plants exhibit a wide range of heights.

Quantitative characteristics arise from two phenomena. First, many are polygenic: they are influenced by genes at many loci. If many loci take part, many genotypes are possible, resulting in many different phenotypes. Second, quantitative characteristics often arise when environmental factors affect the phenotype because environmental variation results in a single genotype producing a range of phenotypes. Most continuously varying characteristics are *both* polygenic *and* influenced by environmental factors, and these characteristics are said to be multifactorial.

The Relation Between Genotype and Phenotype

For some discontinuous characteristics, the relation between genotype and phenotype is straightforward: each genotype produces a single phenotype, and most phenotypes are encoded by a single genotype. Dominance and epistasis may allow different genotypes to produce the same phenotype, but the relation remains simple. This simple relation between genotype and phenotype allowed Mendel to

decipher the basic rules of inheritance from his crosses with pea plants. It also permits us to predict the outcome of genetic crosses and to assign genotypes to individuals.

For quantitative characteristics, the relation between genotype and phenotype is usually more complex. If the characteristic is polygenic, many different genotypes are possible, several of which may produce the same phenotype. For instance, consider a plant whose height is determined by three loci (*A*, *B*, and *C*), each of which has two alleles. Assume that one allele at each locus (A^+, B^+, and C^+) encodes a plant hormone that causes the plant to grow 1 cm above its baseline height of 10 cm. The other allele at each locus (A^-, B^-, and C^-) does not encode a plant hormone and thus does not contribute to additional height. If we consider only the two alleles at a single locus, three genotypes are possible (A^+A^+, A^+A^-, and A^-A^-). If all three loci are taken into account, there are a total of $3^3 = 27$ possible multilocus genotypes ($A^+A^+\ B^+B^+\ C^+C^+$, $A^+A^-\ B^+B^+\ C^+C^+$, etc.). Although there are 27 genotypes, they produce only 7 phenotypes (10 cm, 11 cm, 12 cm, 13 cm, 14 cm, 15 cm, and 16 cm in height) because some of the genotypes produce the same phenotype (**Table 24.1**). For example, genotypes $A^+A^-\ B^-B^-\ C^-C^-$, $A^-A^-\ B^+B^-\ C^-C^-$, and $A^-A^-\ B^-B^-\ C^+C^-$ each have one allele that encodes a plant hormone. Each of these genotypes produces one dose of the hormone and results in a plant that is 11 cm tall. Even in this simple example with only three loci, the relation between genotype and phenotype is quite complex. The more loci encoding a

characteristic, the greater the complexity. As the number of loci encoding a characteristic increases, the number of potential phenotypes increases, and differences between individual phenotypes become more difficult to distinguish.

TABLE 24.1 Hypothetical example of plant height determined by pairs of alleles at each of three loci

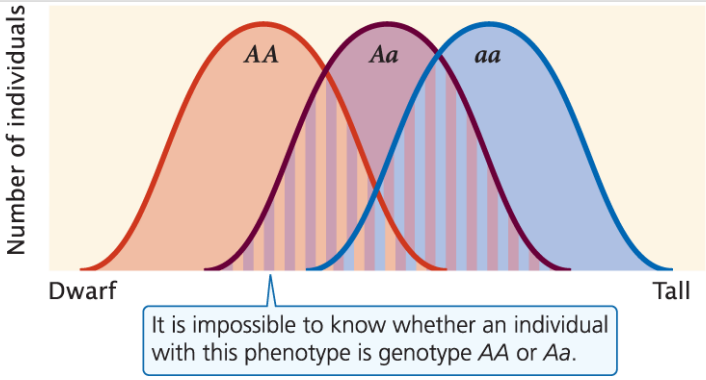
Plant Genotype	Doses of Hormone	Height (cm)
$A^-A^-\ B^-B^-\ C^-C^-$	0	10
$A^+A^-\ B^-B^-\ C^-C^-$	1	11
$A^-A^-\ B^+B^-\ C^-C^-$		
$A^-A^-\ B^-B^-\ C^+C^-$		
$A^+A^+\ B^-B^-\ C^-C^-$	2	12
$A^-A^-\ B^+B^+\ C^-C^-$		
$A^-A^-\ B^-B^-\ C^+C^+$		
$A^+A^-\ B^+B^-\ C^-C^-$		
$A^+A^-\ B^-B^-\ C^+C^-$		
$A^-A^-\ B^+B^-\ C^+C^-$		

$A^+A^+\,B^+B^-\,C^-C^-$	3	13
$A^+A^+\,B^-B^-\,C^+C^-$		
$A^+A^-\,B^+B^+\,C^-C^-$		
$A^-A^-\,B^+B^+\,C^+C^-$		
$A^+A^-\,B^-B^-\,C^+C^+$		
$A^-A^-\,B^+B^-\,C^+C^+$		
$A^+A^-\,B^+B^-\,C^+C^-$		
$A^+A^+\,B^+B^+\,C^-C^-$	4	14
$A^+A^+\,B^+B^-\,C^+C^-$		
$A^+A^-\,B^+B^+\,C^+C^-$		
$A^-A^-\,B^+B^+\,C^+C^+$		
$A^+A^+\,B^-B^-\,C^+C^+$		
$A^+A^-\,B^+B^-\,C^+C^+$		
$A^+A^+\,B^+B^+\,C^+C^-$	5	15
$A^+A^-\,B^+B^+\,C^+C^+$		
$A^+A^+\,B^+B^-\,C^+C^+$		

$A^+A^+\ B^+B^+\ C^+C^+$	6	16

Note: Each + allele contributes 1 cm in height above a baseline of 10 cm.

The influence of environment on a characteristic can also complicate the relation between genotype and phenotype. When environmental factors affect the phenotype, a single genotype can produce a range of phenotypes. Furthermore, the phenotypic ranges of different genotypes can overlap, making it difficult to know whether individuals differ in phenotype because of genetic or environmental differences (Figure 24.2).



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24.2 For a quantitative characteristic influenced by environmental factors, each genotype may produce a range of possible phenotypes. In this hypothetical example, the phenotypes produced by genotypes *AA*, *Aa*, and *aa* overlap.

Description

The horizontal axis represents height: dwarf at the left end and tall at the right end. The vertical axis represents number of individuals. The first bell shaped curve for genotype big A big A starts at dwarf and ends a little after the center of horizontal axis. The second bell shaped curve for genotype big A small A starts a little after origin and ends a little before

tall. The third bell shaped curve starts around the center of the horizontal axis and ends at tall. The overlapping regions of all three curves are shaded. A caption reads, It is impossible to know whether an individual with this phenotype is genotype big A big A or big A small A.

In summary, the simple relation between genotype and phenotype that exists for many qualitative (discontinuous) characteristics is absent for quantitative characteristics, and it is impossible to assign a genotype to an individual on the basis of its phenotype alone. The methods used for analyzing qualitative characteristics (examining the phenotypic ratios of progeny from a genetic cross) will not work for quantitative characteristics. Our goal remains the same: we wish to make predictions about the phenotypes of offspring produced in a genetic cross. We may also want to know how much of the variation in a characteristic results from genetic differences and how much results from environmental differences. To answer these questions, we must turn to statistical methods that allow us to make predictions about the inheritance of phenotypes in the absence of information about the underlying genotypes. › **TRY** PROBLEM 16

Types of Quantitative Characteristics

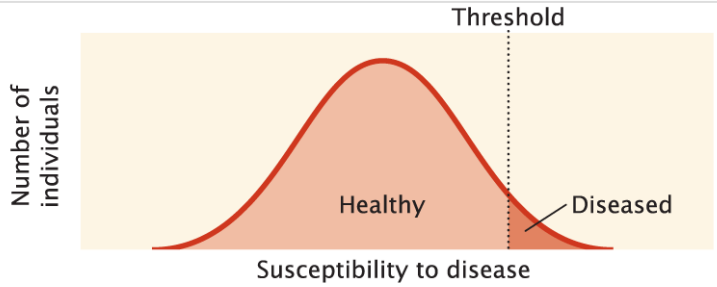
Before we look more closely at polygenic characteristics and relevant statistical methods, we need to more clearly define what is meant by a quantitative characteristic. Thus far, we have considered only quantitative characteristics that vary continuously in a

population. A *continuous characteristic* can theoretically assume any value between two extremes; the number of phenotypes is limited only by our ability to precisely measure them. Human height is a continuous characteristic because, within certain limits, people can theoretically have any height. Although the number of phenotypes possible with a continuous characteristic is infinite, we often group similar phenotypes together for convenience; we may say that two people are both 5 feet 11 inches tall, although careful measurement may show that one is slightly taller than the other.

Some characteristics are not continuous but are nevertheless considered quantitative because they are determined by multiple genetic and environmental factors. **Meristic characteristics**, for instance, are measured in whole numbers. An example is litter size: a female mouse can have 4, 5, or 6 pups, but not 4.13 pups. A meristic characteristic has a limited number of distinct phenotypes, but the underlying determination of the characteristic is still quantitative. These characteristics must therefore be analyzed with the same techniques that we use to study continuous quantitative characteristics.

Another type of quantitative characteristic is a **threshold characteristic**, which is simply present or absent. Although threshold characteristics exhibit only two phenotypes, they are considered quantitative because they also are determined by multiple genetic and environmental factors. The expression of the characteristic depends on an underlying susceptibility (usually

referred to as liability or risk) that varies continuously. When the susceptibility is larger than a threshold value, a specific trait is expressed (Figure 24.3). Diseases are often threshold characteristics because many factors, both genetic and environmental, contribute to disease susceptibility. If enough of the susceptibility factors are present, the disease develops; otherwise, it is absent. Although we focus on the genetics of continuous characteristics in this chapter, the same principles apply to many meristic and threshold characteristics.



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24.3 Threshold characteristics display only two possible phenotypes—the trait is either present or absent—but they are quantitative because the underlying susceptibility to the characteristic varies continuously. When the susceptibility exceeds a threshold value, the characteristic is expressed.

Description

The horizontal axis represents susceptibility to disease. The vertical axis represents number of individuals. A bell shaped curve starts a little after origin and ends towards the end of the horizontal axis. The curve peaks at the center of the graph. The area underneath the curve is labeled healthy. Near the right end of the graph as the curve is tapering off, a dashed line is labeled threshold. To the right of this dashed line, the area underneath the curve is labeled diseased.

Just because a characteristic can be measured on a continuous scale does not mean that it exhibits quantitative variation. One of the characteristics studied by Mendel was the height of pea plants, which can be described by measuring the length of a plant's stem. However, Mendel's particular plants exhibited only two distinct phenotypes (some were tall and others short), and these differences were determined by alleles at a single locus. The differences that Mendel studied were therefore discontinuous in nature.

CONCEPTS

Characteristics for which the phenotypes vary continuously are quantitative characteristics. For most quantitative characteristics, the relation between genotype and phenotype is complex. Some characteristics for which the phenotypes do not vary continuously are also considered quantitative because they are influenced by multiple genes and environmental factors.

Polygenic Inheritance

After the rediscovery of Mendel's work in 1900, questions soon arose about the inheritance of continuously varying characteristics. These characteristics had already been the focus of a group of biologists and statisticians, led by Francis Galton, who used statistical procedures to examine the inheritance of characteristics such as human height and intelligence. The results of these studies showed that quantitative characteristics were at least partly inherited,

although the mechanism of inheritance was not yet known. Some biometricians argued that the inheritance of quantitative characteristics could not be explained by Mendelian principles, whereas others believed that Mendel's principles acting on numerous genes (polygenes) could adequately account for the inheritance of quantitative characteristics.

This conflict began to be resolved by the work of Wilhelm Johannsen, who showed that continuous variation in the weight of beans was influenced by both genetic and environmental factors. George Udny Yule, a mathematician, proposed in 1906 that several genes acting together could produce continuous characteristics. This hypothesis was later confirmed by Herman Nilsson-Ehle, working on wheat and tobacco, and by Edward East, working on corn. The argument was finally laid to rest in 1918, when Ronald Fisher demonstrated that the inheritance of quantitative characteristics could indeed be explained by the cumulative effects of many genes, each following Mendel's rules.

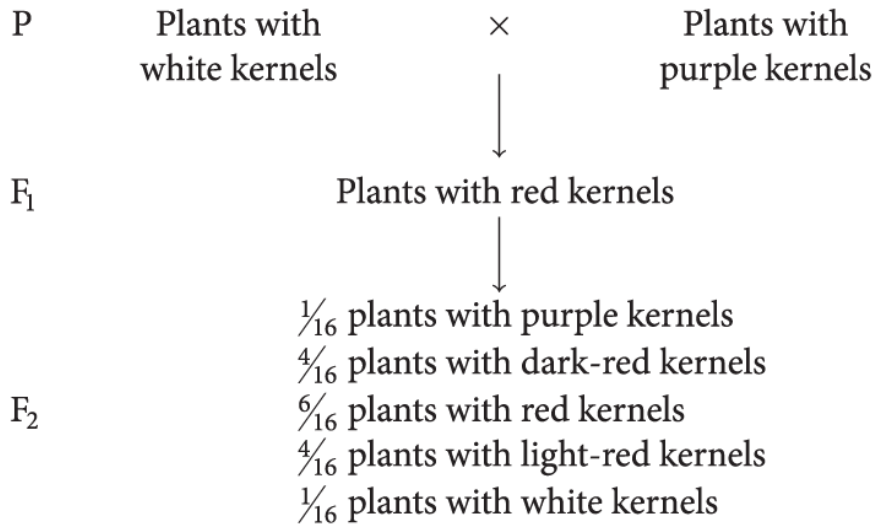
Kernel Color in Wheat

To illustrate how multiple genes acting on a characteristic can produce a continuous range of phenotypes, let's examine one of the first demonstrations of polygenic inheritance. Nilsson-Ehle studied kernel color in wheat and found that the intensity of red

pigmentation was determined by three unlinked loci, each of which had two alleles.

NILSSON-EHLE'S CROSS

Nilsson-Ehle obtained several homozygous varieties of wheat that differed in color. Like Mendel, he performed crosses between these homozygous varieties and studied the ratios of phenotypes in the progeny. In one experiment, he crossed a variety of wheat that possessed white kernels with a variety that possessed purple (very dark red) kernels and obtained the following results:



Description
The P generation at the top shows plants with white kernels crossed with plants with purple kernels. The F subscript 1 generation below shows plants with red kernels. The G subscript 2 generation obtained below shows the following: one-sixteenth plants with purple kernels, four-sixteenth plants with dark-red kernels, six-sixteenth plants with red kernels, four-sixteenth plants with light-red kernels, and one-sixteenth plants with white kernels.

INTERPRETATION OF THE CROSS

Nilsson-Ehle interpreted this phenotypic ratio as the result of the segregation of alleles at two loci. (Although he found alleles at three loci that affect kernel color, the two varieties used in this cross differed at only two of the loci.) He proposed that there were two alleles at each locus: one that produced red pigment and another that produced no pigment. We'll designate the alleles that encoded pigment A^+ and B^+ and the alleles that encoded no pigment A^- and B^-. Nilsson-Ehle recognized that the effects of the genes were additive. Each gene seemed to contribute equally to color, so the overall phenotype could be determined by adding the effects of all the alleles, as shown in the following table:

Genotype	Doses of pigment	Phenotype
$A^+A^+\,B^+B^+$	4	Purple
$A^+A^+\,B^+B^-$ $A^+A^-\,B^+B^+$	3	Dark red
$A^+A^+\,B^-B^-$ $A^-A^-\,B^+B^+$ $A^+A^-\,B^+B^-$	2	Red
$A^+A^-\,B^-B^-$ $A^-A^-\,B^+B^-$	1	Light red
$A^-A^-\,B^-B^-$	0	White

Notice that the purple and white phenotypes are each encoded by a single genotype, but the other phenotypes may result from several different genotypes.

From these results, we see that five phenotypes are possible when alleles at two loci influence the phenotype and the effects of the genes are additive. If alleles at more than two loci influenced the phenotype, more phenotypes would be possible, and the color would appear to vary continuously between white and purple. If environmental factors influenced the characteristic, individuals of the same genotype would vary somewhat in color, making it even more difficult to distinguish between discrete phenotypic classes. Luckily, environment played little role in determining kernel color in Nilsson-Ehle's crosses, and only a few loci encoded color, so Nilsson-Ehle was able to distinguish among the different phenotypic classes. This ability allowed him to see the Mendelian nature of the characteristic.

Let's now see how Mendel's principles explain the ratio obtained by Nilsson-Ehle in his F_2 progeny. Remember that Nilsson-Ehle crossed the homozygous purple variety ($A^+A^+\ B^+B^+$) with the homozygous white variety ($A^-A^-\ B^-B^-$), producing F_1 progeny that were heterozygous at both loci ($A^+A^-\ B^+B^-$). This is a dihybrid cross, like those that we worked in Chapter 3, except that both loci encode the same trait. Each of the F_1 plants possessed two pigment-producing alleles, so each plant received two doses of pigment, which

produced red kernels. The types and proportions of progeny expected in the F_2 can be found by applying Mendel's principles of segregation and independent assortment.

Let's first examine the effects of each locus separately. At the first locus, two heterozygous F_1s are crossed ($A^+A^- \times A^+A^-$). As we learned in Chapter 3, when two heterozygotes are crossed, we expect progeny in the proportions $^1/_4\ A^+A^+$, $^1/_2\ A^+A^-$, and $^1/_4\ A^-A^-$. At the second locus, two heterozygotes are also crossed, and again we expect progeny in the proportions $^1/_4\ B^+B^+$, $^1/_2\ B^+B^-$, and $^1/_4\ B^-B^-$.

To obtain the probability of combinations of genes at both loci, we must use the multiplication rule (see Chapter 3), the use of which assumes Mendel's principle of independent assortment. The expected proportion of F_2 progeny with genotype $A^+A^+\ B^+B^+$ is the product of the probability of obtaining genotype A^+A^+ ($^1/_4$) and the probability of obtaining genotype B^+B^+ ($^1/_4$), or $^1/_4 \times {}^1/_4 = {}^1/_{16}$ (**Figure 24.4**). The probabilities of each of the phenotypes can then be obtained by adding the probabilities of all the genotypes that produce that phenotype. For example, the red phenotype is produced by three genotypes:

Genotype	Phenotype	Probability
$A^+A^+\ B^-B^-$	Red	$^1/_{16}$
$A^-A^-\ B^+B^+$	Red	$^1/_{16}$

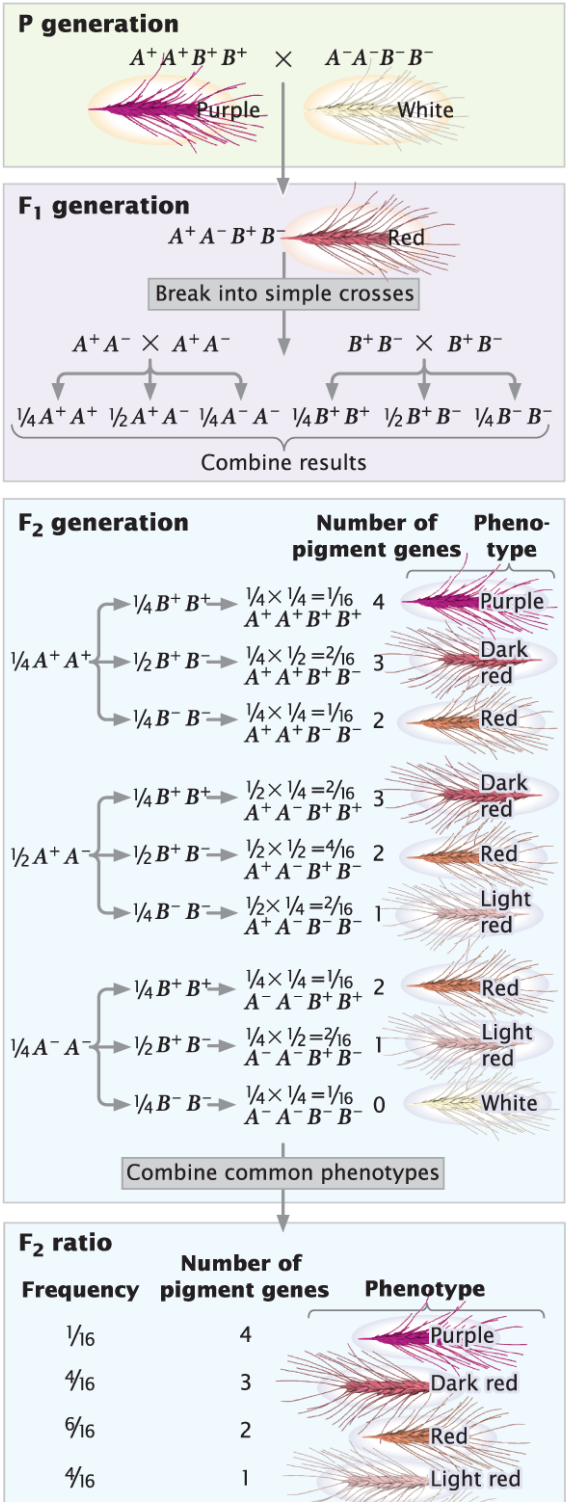
$A^{+}A^{-}\,B^{+}B^{-}$	Red	$^{1}/_{4}$

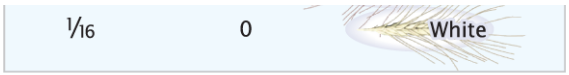
Experiment

Question: How is a continuous trait, such as kernel color in wheat, inherited?

Methods Cross wheat with white kernels and wheat with purple kernels. Intercross the F_1 to produce F_2.

Results





Conclusion: Kernel color in wheat is inherited according to Mendel's principles acting on alleles at two loci.

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24.4 Nilsson-Ehle demonstrated that kernel color in wheat is inherited according to Mendelian principles. The ratio of phenotypes in the F_2 can be determined by breaking the dihybrid cross into two simple single-locus crosses and combining the results by using the multiplication rule.

Description

The illustration begins with a question that reads, How is a continuous trait, such as kernel color in wheat, inherited? The methods below show cross wheat with white kernels and wheat with purple kernels. Intercross the F subscript 1 to produce F subscript 2. P generation below shows wheat with purple kernels big A superscript positive big A superscript positive big B superscript positive big B superscript positive is crossed with white kernels big A superscript negative big A superscript negative big B superscript negative big B superscript negative. The results section below shows F subscript 1 generation. The F subscript 1 generation shows wheat with red kernels with genotype big A superscript positive big A superscript negative big B superscript positive big B superscript negative. Breaking into simple crosses occurs below. The first cross on the left shows: big A superscript positive big A superscript negative crossed with big A superscript positive big A superscript negative. The results below are: one-fourth big A superscript positive big A superscript positive, one-half big A superscript positive big A superscript negative, and one-fourth big A superscript negative big A superscript negative.

The second cross on the right shows: big B superscript positive big B superscript negative crossed with big B superscript positive big B superscript negative. The results below are: one-fourth big B superscript positive big B superscript positive, one-half big B superscript positive big B superscript negative, and one-fourth big B superscript negative big B superscript negative. The results are combined and F subscript 2 generation is obtained below.

The F subscript 2 generation is in the form of a table. The table shows 3 rows and 3 columns. The column headers are as follows: F subscript 2 generation, number of

pigment genes, and phenotype.

Row entries are as follows:

Row 1: one-fourth big A superscript positive big A superscript positive with one-fourth big B superscript positive big B superscript positive gives one-fourth big A superscript positive big A superscript positive multiplied by one-fourth big B superscript positive big B superscript positive equals one-sixteenth; 4; purple.

one-fourth big A superscript positive big A superscript positive with one-half big B superscript positive big B superscript negative gives one-fourth big A superscript positive big A superscript positive multiplied by one-half big B superscript positive big B superscript negative equals two-sixteenth; 3; dark red.

one-fourth big A superscript positive big A superscript positive with one-fourth big B superscript negative big B superscript negative gives one-fourth big A superscript positive big A superscript positive multiplied by one-fourth big B superscript negative big B superscript negative equals one-sixteenth; 2; red.

Row 2: one-half big A superscript positive big A superscript negative with one-fourth big B superscript positive big B superscript positive gives one-half big A superscript positive big A superscript negative multiplied by one-fourth big B superscript positive big B superscript positive equals two-sixteenth; 3; dark red.

one-half big A superscript positive big A superscript negative with one-half big B superscript positive big B superscript negative gives one-half big A superscript positive big A superscript negative multiplied by one-half big B superscript positive big B superscript negative equals four-sixteenth; 2; red.

one-half big A superscript positive big A superscript negative with one-fourth big B superscript negative big B superscript negative gives one-half big A superscript positive big A superscript negative multiplied by one-fourth big B superscript negative big B superscript negative equals two-sixteenth; 1; light red.

Row 3: one-fourth big A superscript negative big A superscript negative with one-fourth big B superscript positive big B superscript positive gives one-fourth big A superscript negative big A superscript negative multiplied by one-fourth big B superscript positive big B superscript positive equals equals one-sixteenth; 2; red.

one-fourth big A superscript negative big A superscript negative with one-half big B superscript positive big B superscript negative gives one-fourth big A superscript negative big A superscript negative multiplied by one-half big B superscript positive big B superscript negative equals equals two-sixteenth; 1; light red.

one-fourth big A superscript negative big A superscript negative with one-fourth big B superscript negative big B superscript negative gives one-fourth big A superscript negative big A superscript negative multiplied by one-fourth big B superscript negative big B superscript negative equals one-sixteenth; 0; white. Combine common phenotypes and a table with 3 columns and 5 rows is obtained below.

The column headers are as follows: F subscript 2 ratio Frequency, Number of pigment genes, Phenotype. The row entries are as follows:

Row 1: one-sixteenth; 4; Purple.

Row 2: four-sixteenth; 3; Dark red.

Row 3: six-sixteenth; 2; Red.

Row 4: four-sixteenth; 1; Light red.

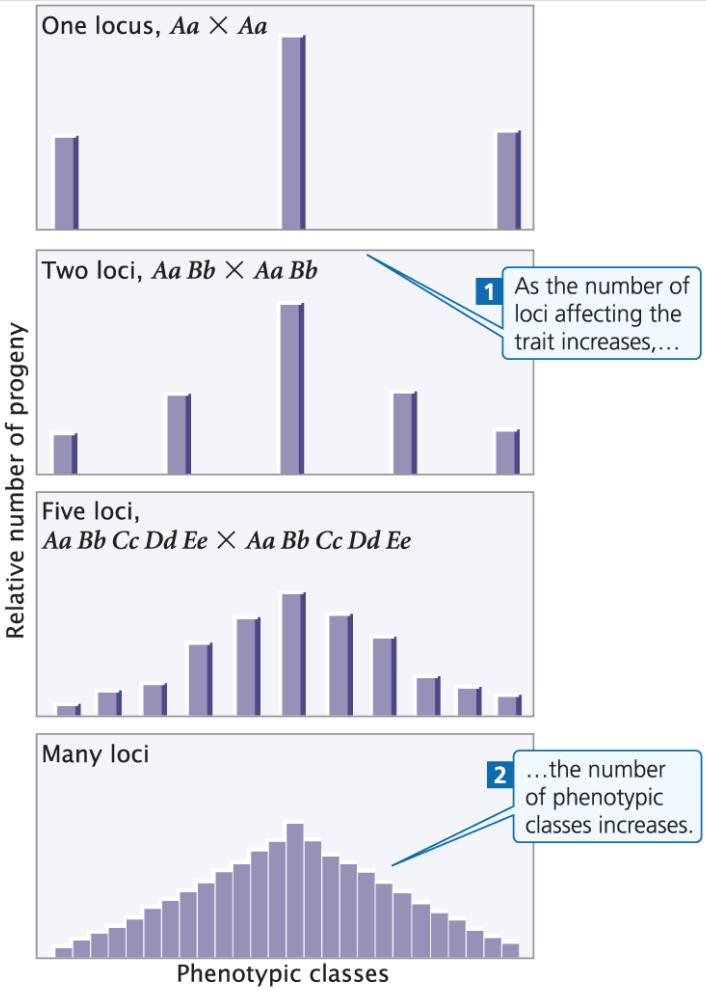
Row 5: one-sixteenth; 0; white.

A caption below reads, Conclusion: Kernel color in wheat is inherited according to Mendel's principles acting on alleles at two loci.

Thus, the overall probability of obtaining red kernels in the F_2 progeny is $^1/_{16} + {}^1/_{16} + {}^1/_4 = {}^6/_{16}$. Figure 24.4 shows that the phenotypic ratio expected in the F_2 is $^1/_{16}$ purple, $^4/_{16}$ dark red, $^6/_{16}$ red, $^4/_{16}$ light red, and $^1/_{16}$ white. This phenotypic ratio is precisely what Nilsson-Ehle observed in his F_2 progeny, demonstrating that the inheritance of a continuously varying characteristic, such as kernel color, is indeed explained by Mendel's basic principles.

CONCLUSIONS AND IMPLICATIONS

Nilsson-Ehle's crosses demonstrated that the difference between the inheritance of genes influencing quantitative characteristics and the inheritance of genes influencing discontinuous characteristics is in the *number* of loci that determine the characteristic. When multiple loci affect a characteristic, more genotypes are possible, so the relation between the genotype and the phenotype is less obvious. As the number of loci affecting a characteristic increases, the number of phenotypic classes in the F_2 increases (**Figure 24.5**).



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24.5 Crossing heterozygotes for different numbers of loci affecting a characteristic results in different numbers of phenotypic classes.

Description

All the graphs have a different number of bars, but all four have the same horizontal and vertical axis. Horizontal axes represent phenotypic classes and vertical axes represent relative number of progeny. First bar graph at the top shows that for a cross between big A small A and big A small A (one locus), there are 3 phenotypic classes. The bar at the center is highest and the bar on each side of the horizontal axis is smaller. A caption reads, As the number of loci affecting the trait increases. The second bar graph below shows a cross between big A small A big B small B and big A small A big B small B (two loci). This graph shows 5 phenotypic classes. The bars on the left gradually increase such

that the peak at the center is highest and then gradually reduces on the right side. The third bar graph below shows big A small A big B small B big C small C big D small D big E small E crossed with big A small A big B small B big C small C big D small D big E small E (five loci). These progeny have 11 phenotypic classes. The bars on the left gradually increase such that the peak at the center is highest and then gradually decrease on the right side. The fourth bar graph below is labeled many loci. It has maximum number of phenotypic classes. The bars on the left gradually increase such that the peak at the center is highest and then gradually decrease on the right side. A caption reads, the number of phenotypic classes increases.

Several conditions of Nilsson-Ehle's crosses greatly simplified the polygenic inheritance of kernel color and made it possible for him to recognize the Mendelian nature of the characteristic. First, genes affecting color segregated at only two or three loci. If genes at many loci had been segregating, he would have had difficulty distinguishing the phenotypic classes. Second, the genes affecting kernel color had strictly additive effects, making the relation between genotype and phenotype simple. Third, environment played almost no role in the phenotype; had environmental factors modified the phenotypes, distinguishing between the five phenotypic classes would have been difficult. Finally, the loci that Nilsson-Ehle studied were not linked, so the genes assorted independently. Nilsson-Ehle was fortunate: for many polygenic characteristics, these simplifying conditions are not present, and the Mendelian inheritance of these characteristics is not obvious. › **TRY PROBLEM 18**

THINK-PAIR-SHARE Question 1

Determining Gene Number for a Polygenic Characteristic

The proportion of F_2 individuals that resemble one of the original parents can be used to estimate the number of genes affecting a polygenic trait. When two individuals homozygous for different alleles at a single locus are crossed ($A^1A^1 \times A^2A^2$) and the resulting F_1 are interbred ($A^1A^2 \times A^1A^2$), one-fourth of the F_2 should be homozygous like each of the original parents. If the original parents are homozygous for different alleles at two loci, as are those in Nilsson-Ehle's crosses, then $^1/_4 \times {}^1/_4 = {}^1/_{16}$ of the F_2 should resemble one of the original homozygous parents. Generally, $(^1/_4)^n$ will be the proportion of individuals among the F_2 progeny that should resemble each of the original homozygous parents, where n equals the number of loci with a segregating pair of alleles that affects the characteristic. This equation provides us with a possible means of determining the number of loci influencing a quantitative characteristic.

To illustrate the use of this equation, assume that we cross two different homozygous varieties of pea plants that differ in height by 16 cm, interbreed the F_1, and find that approximately $^1/_{256}$ of the F_2 are similar to one of the original homozygous parental varieties. This outcome would suggest that four loci with segregating pairs of alleles ($^1/_{256} = (^1/_4)^4$) are responsible for the height difference between the two varieties. Because the two homozygous strains

differ in height by 16 cm and there are four loci, each of which has two alleles (eight alleles in all), each of the alleles contributes 16 cm/8 = 2 cm in height.

This method of determining the number of loci affecting phenotypic differences requires the use of homozygous strains, which may be difficult to obtain in some organisms. It also assumes that all the genes influencing the characteristic have equal effects, that their effects are additive, and that the loci are unlinked. For many polygenic characteristics, these assumptions are not valid, and so this method of determining the number of genes affecting a characteristic has limited application. › **TRY** PROBLEM 20

CONCEPTS

The same principles determine the inheritance of quantitative and discontinuous characteristics, but more genes take part in the determination of quantitative characteristics.

✔ CONCEPT CHECK 1

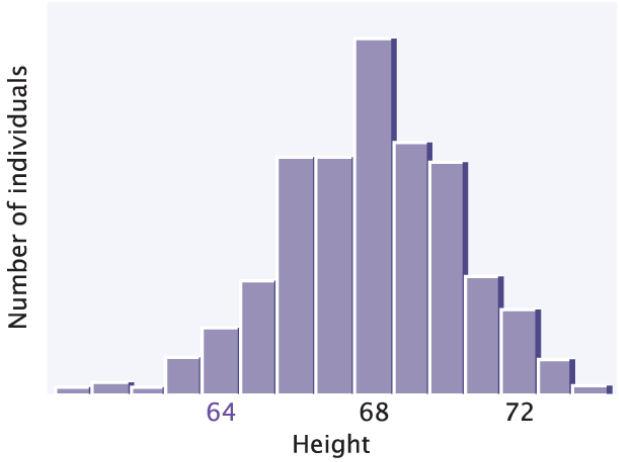
Briefly explain how the number of genes influencing a polygenic trait can be determined.

24.2 Statistical Methods Are Required for Analyzing Quantitative Characteristics

Because quantitative characteristics are described by a measurement and are influenced by multiple factors, their inheritance must be analyzed statistically. This section explains the basic concepts of statistics that are used to analyze quantitative characteristics.

Distributions

An understanding of the genetic basis of any characteristic begins with a description of the numbers and kinds of phenotypes present in a group of individuals. Phenotypic variation in a group can be conveniently represented by a **frequency distribution**, which is a graph of the frequencies (numbers or proportions) of the different phenotypes (Figure 24.6). In a typical frequency distribution, phenotypic classes are plotted on the horizontal (x) axis, and the numbers (or proportions) of individuals in each class are plotted on the vertical (y) axis. A frequency distribution is a concise method of summarizing all phenotypes of a quantitative characteristic.

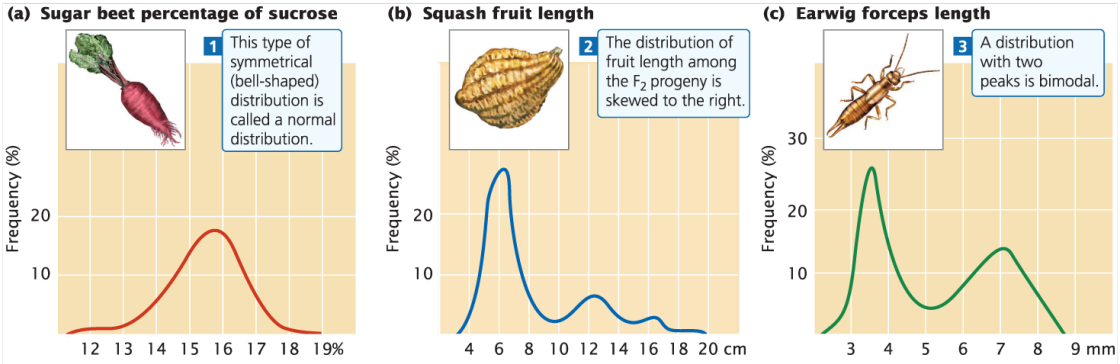


24.6 A frequency distribution is a graph that displays the numbers or proportions of different phenotypes present in a group of individuals. Phenotypic values (here, height in inches) are plotted on the horizontal axis, and the numbers (or proportions) of individuals with each phenotype are plotted on the vertical axis.

Description

The horizontal axis represents height with the following points: 64, 68, 72. The vertical axis represents number of individuals. The bars gradually increase from the left side and peak above point 68, then gradually decrease as they reach the right end of the graph.

Connecting the points of a frequency distribution with a line creates a curve that is characteristic of the distribution. Many quantitative characteristics exhibit a symmetrical (bell-shaped) curve called a **normal distribution** (Figure 24.7a). Normal distributions arise when a large number of independent factors contribute to a measurement, as is often the case in quantitative characteristics. Two other common types of distributions (skewed and bimodal) are illustrated in Figure 24.7b and c.



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24.7 Distributions of phenotypes can assume several different shapes.

Description

Graph a is labeled sugar beet percentage of sucrose. The horizontal axis ranges from 12 to 19 percent, in increments of 1. The vertical axis represents frequency percent and ranges from 10 to 20, in increments of 10. A bell shaped curve starts before (12, 0), peaks at (15 point 5, 17), and then finally reaches (19, 0). A photo of beet is shown on the top left corner. A caption reads, This type of symmetrical (bell-shaped) distribution is called a normal distribution.

Graph b is labeled Squash fruit length. The horizontal axis ranges from 4 to 20 c m, in increments of 2. The vertical axis represents frequency percent and ranges from 10 to 20, in increments of 10. A curve starts at (4, 0), peaks at (7, 30), reaches (10, 2), (13, 1) before finally reaching (20, 0). A photo of squash is shown on the top left corner. A caption reads, The distribution of fruit length among the F subscript 2 progeny is skewed to the right.

Graph c is labeled Earwig forceps length. The horizontal axis ranges from 3 to 9 m m, in increments of 1. The vertical axis represents frequency percent and ranges from 10 to 30, in increments of 10. A curve starts at (3, 0), peaks at (4, 26), reaches (5 point 5, 5), (7 point 5, 13) before finally reaching (9, 0). A photo of an earwig is shown on the top left corner. A caption reads, A distribution with two peaks is bimodal.

All data are approximate.

Samples and Populations

Biologists frequently need to describe the distribution of phenotypes exhibited by a group of individuals. We might want to describe the height of students at the University of Texas (UT), but UT has more than 40,000 students, and measuring all of them is not practical. Scientists are constantly confronted with this problem: the group of interest, called the **population**, is too large for a complete census. One solution is to measure a smaller collection of individuals, called a **sample**, and use those measurements to describe the population.

To provide an accurate description of the population, a good sample must have several characteristics. First, it must be representative of the whole population—for instance, if our sample consisted entirely of members of the UT basketball team, we would probably overestimate the true height of the students. One way to ensure that a sample is representative of the population is to select the members of the sample randomly. Second, the sample must be large enough that chance differences between the individuals in the sample and the overall population do not distort the estimate of the population measurements. If we measured only three students at UT and just by chance all three were short, we would underestimate the true height of the student population. Statistics can tell us how much confidence to have in estimates based on random samples.

THINK-PAIR-SHARE Question 2

CONCEPTS

In statistics, the population is the group of interest; a sample is a subset of the population. The sample should be representative of the population and large enough to minimize chance differences between the population and the sample.

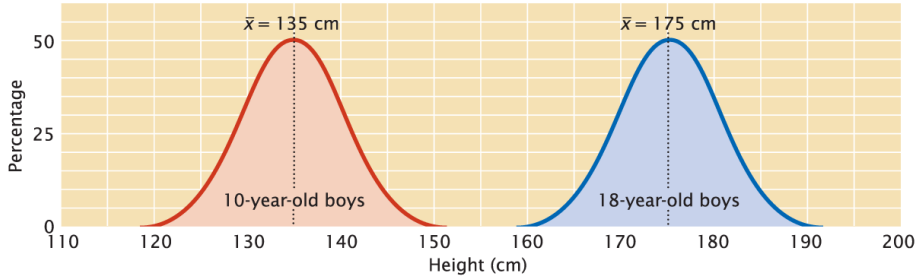
✔ CONCEPT CHECK 2

A geneticist is interested in whether asthma is caused by a mutation in the *DS112* gene. The geneticist collects DNA from 120 people with asthma and 100 healthy people, and she sequences the DNA. She finds that 35 of the people with asthma and none of the healthy people have a mutation in the *DS112* gene. What is the population in this study?

a. The 120 people with asthma
b. The 100 healthy people
c. The 35 people with a mutation in the *DS112* gene
d. All people with asthma

The Mean

The **mean**, also called the average, is a statistic that provides information about the center of a distribution. If we measured the heights of 10-year-old and 18-year-old boys and plotted a frequency distribution for each group, we would find that both distributions are normal, but the two distributions would be centered at different heights, and this difference would be indicated by their different means (**Figure 24.8**).



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24.8 The mean provides information about the center of a distribution. The distributions of the heights of both 10-year-old and 18-year-old boys are normal, but they have different locations along a continuum of height, which makes their means different.

Description
The horizontal axis represents height (c m) and ranges from 110 to 200, in increments of 10. The vertical axis represents percentage and ranges from 0 to 50, in increments of 25. A bell shaped curve for 10 year old boys starts at (120, 0), peaks at (135, 50), and ends at (50, 0). The peak is labeled x bar equals 135 c m. A bell shaped curve for 18 year old boys starts at (160, 0), peaks at (175, 50), and ends at (190, 0). The peak is labeled x bar equals 175 c m. All data are approximate.

Suppose that we have five measurements of height in centimeters: 160, 161, 167, 164, and 165. If we represent a group of measurements as x_1, x_2, x_3, and so forth, then the mean (x) is calculated by adding all the individual measurements and dividing by the total number of measurements in the sample (n):

$$x = \frac{x_1 + x_2 + x_3 + \cdots + x_n}{n} \tag{24.1}$$

In our example, x_1 = 160, x_2 = 161, x_3 =167, and so forth. The mean height (x) equals

$$x = \frac{160 + 161 + 167 + 164 + 165}{5} = \frac{817}{5} = 163.4$$

A shorthand way to represent this formula is

$$x = \frac{\sum x_i}{n} \tag{24.2}$$

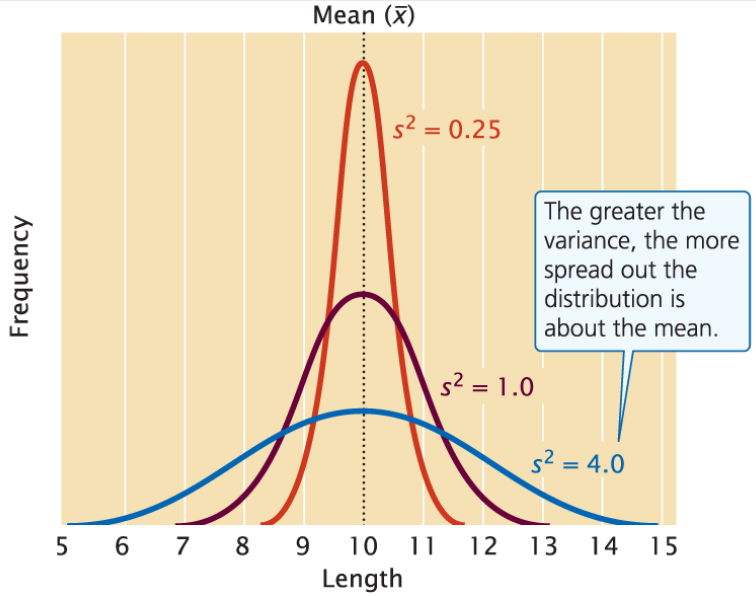
or

$$x = \frac{1}{n} \sum x_i \tag{24.3}$$

where the symbol Σ means “the summation of” and x_i represents the individual x values.

The Variance and Standard Deviation

A statistic that provides key information about a distribution is its **variance**, which indicates the variability of a group of measurements, or how spread out the distribution is. Distributions can have the same mean but different variances (**Figure 24.9**). The larger the variance, the greater the spread of measurements in a distribution about its mean.



24.9 The variance provides information about the variability of a group of phenotypes. Shown here are three distributions with the same mean but different variances.

Description
The horizontal axis represents length and ranges from 5 to 15, in increments of 1. The vertical axis represents frequency. A bell shaped curve for s squared equals 4 point 0 starts at 5 on the horizontal axis, peaks at 10 and ends at 15. A caption reads, The greater the variance, the more spread out the distribution is about the mean. A bell shaped curve for s squared equals 1 point 0 starts at 7 on the horizontal axis, peaks at 10 and ends at 13. A bell shaped curve for s squared equals 0 point 2 5 starts at 8 point 3 on the horizontal axis, peaks at 10 and ends at 11 point 8. All data are approximate.

The variance (s^2) is defined as the average squared deviation from the mean:

$$s^2 = \frac{\sum (x_i - x)^2}{n - 1} \tag{24.4}$$

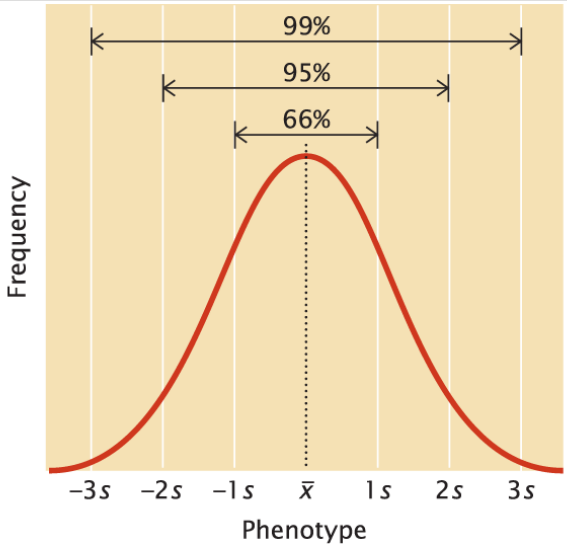
To calculate the variance, we (1) subtract the mean from each measurement and square the value obtained, (2) add all of these squared deviations together, and (3) divide that sum by the number of original measurements minus 1.

Another statistic that is closely related to the variance is the **standard deviation** (s), which is defined as the square root of the variance:

$$s = \sqrt{s^2} \tag{24.5}$$

Whereas the variance is expressed in units squared, the standard deviation is in the same units as the original measurements; thus, the standard deviation is often preferred for describing the variability of a measurement.

Because a normal distribution is symmetrical, its mean and standard deviation are sufficient to describe its shape. The mean plus or minus one standard deviation ($x \pm s$) includes approximately 66% of the measurements in a normal distribution; the mean plus or minus two standard deviations ($x \pm 2s$) includes approximately 95% of the measurements; and the mean plus or minus three standard deviations ($x \pm 3s$) includes approximately 99% of the measurements (**Figure 24.10**). Thus, only 1% of a normally distributed population lies outside the range of ($x \pm 3s$). ❯ **TRY PROBLEM 24**



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24.10 The proportions of a normal distribution occupied by plus or minus one, two, and three standard deviations from the mean.

Description
The horizontal axis represents phenotype and has the following points: negative 3 s, negative 2 s, negative 1 s, x bar, 1 s, 2 s, and 3 s. The vertical axis represents frequency. A bell shaped curve starts before negative 3 s on the horizontal axis, peaks at x bar, and ends after 3 s. The horizontal distance between negative 1 s and 1 s is labeled 66 percent. The horizontal distance between negative 2 s and 2 s is labeled 95 percent. The horizontal distance between negative 3 s and 3 s is labeled 99 percent. All data are approximate.

CONCEPTS

The mean and the variance are statistics that describe a distribution of measurements: the mean provides information about the location of the center of a distribution, and the variance provides information about its variability.

✔ CONCEPT CHECK 3

The measurements of a distribution with a higher _____ will be more spread out.

a. mean
b. variance
c. standard deviation
d. variance and standard deviation

Correlation

The mean and the variance can be used to describe an individual characteristic, but geneticists are frequently interested in more than one characteristic. Often, two or more characteristics vary together. For instance, both the number and the weight of eggs produced by hens are important to the poultry industry. These two characteristics are not independent of each other. There is an inverse relation between egg number and weight: hens that lay more eggs produce smaller eggs. This kind of relation between two characteristics is called a **correlation**. When two characteristics are correlated, a change in one characteristic is likely to be associated with a change in the other.

Correlations between characteristics are measured by a **correlation coefficient** (designated *r*), which measures the strength of their association. Consider two characteristics, such as human height (*x*) and arm length (*y*). To determine how these characteristics are correlated, we first obtain the covariance (cov) of *x* and *y*:

$$\text{cov}_{xy} = \frac{\sum(x_i - x)(y_i - y)}{n - 1}$$

(24.6)

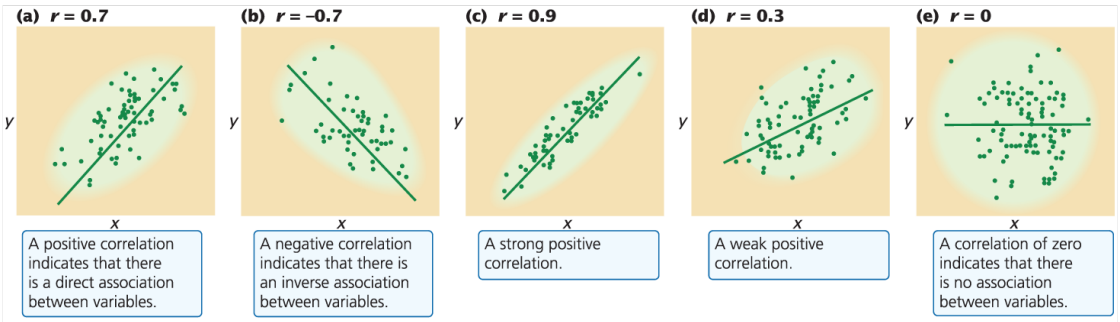
The covariance is computed by (1) taking the *x* value for an individual and subtracting from it the mean of x (x); (2) taking the *y* value for the same individual and subtracting from it the mean of y (y); (3) multiplying the results of these two subtractions; (4) adding the results for all the *xy* pairs; and (5) dividing this sum by $n - 1$ (where *n* equals the number of *xy* pairs).

The correlation coefficient (*r*) is obtained by dividing the covariance of *x* and *y* by the product of the standard deviations of *x* and *y*:

$$r = \frac{\text{cov}_{xy}}{s_x s_y}$$

(24.7)

A correlation coefficient can theoretically range from −1 to +1. A positive value indicates that there is a direct association between the variables (**Figure 24.11a**): as one variable increases, the other variable also tends to increase. A positive correlation exists for human height and weight: tall people tend to weigh more. A negative correlation coefficient indicates that there is an inverse relation between the two variables (**Figure 24.11b**): as one variable increases, the other tends to decrease (as is the case for egg number and egg weight in chickens).



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24.11 The correlation coefficient describes the relation between two or more variables.

Description

All the horizontal axes are labeled x and all the vertical axes are labeled y. Scatter plot a labeled r equals 0 point 7 shows a line with positive slope. The dots are spread in a small area around the line. A caption reads, A positive correlation indicates that there is a direct association between variables.

Scatter plot b labeled r equals negative 0 point 7 shows a line with negative slope. The dots are spread in a large area around the line. A caption reads, A negative correlation indicates that there is an inverse association between variables.

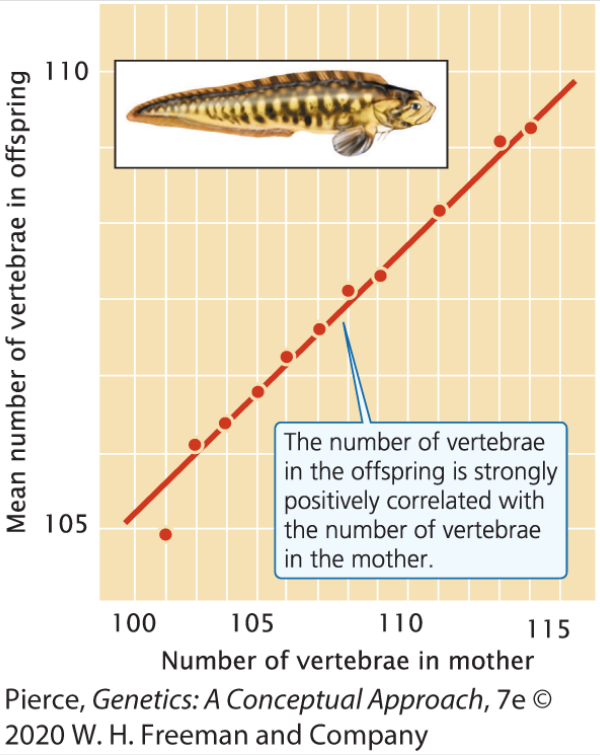
Scatter plot c labeled r equals 0 point 9 shows a line with a positive slope. The dots are very close to the line. A caption reads, A strong positive correlation.

Scatter plot d labeled r equals 0 point 3 shows a line with a shallow positive slope. The dots are spread in a large area around the line. A caption reads, A weak positive correlation.

Scatter plot e labeled r equals 0 shows a horizontal line. The dots are spread in a very large area around the line. A caption reads, A correlation of zero indicates that there is no association between variables.

The absolute value of the correlation coefficient (the size of the coefficient, ignoring its sign) provides information about the strength of association between the variables. A coefficient of −1 or +1 indicates a perfect correlation between the variables, meaning that a change in x is always accompanied by a proportional change in y. Correlation coefficients close to −1 or close to +1 indicate a strong association between the variables: a change in x is almost always associated with a proportional change in y, as seen in Figure 24.11c. On the other hand, a correlation coefficient closer to 0 indicates a weak correlation: a change in x is associated with a change in y, but not always (Figure 24.11d). A correlation of 0 indicates that there is no association between the variables (Figure 24.11e).

A correlation coefficient can be computed for two variables measured for the same individual, such as height (x) and weight (y). A correlation coefficient can also be computed for a single variable measured for pairs of individuals. For example, we can calculate for a fish species the correlation between the number of vertebrae of a parent (x) and the number of vertebrae of its offspring (y), as shown in Figure 24.12. This approach is often used in quantitative genetics.



24.12 A correlation coefficient can be computed for a single variable measured for pairs of individuals. Here, the numbers of vertebrae in mothers and offspring of the fish *Zoarces viviparus* are compared.

Description
The horizontal axis represents number of vertebrae in mother and ranges from 100 to 115, in increments of 5. The vertical axis represents mean number of vertebrae in offspring and ranges from 105 to 110, in increments of 5. A line with positive slope starts from (100, 105) and ends at (115, 109). Large number of dots appears on the line while a single dot is present at (101, 105). A caption reads, The number of vertebrae in the offspring is strongly positively correlated with the number of vertebrae in the mother. A photo shows a fish named Zoarces viviparous at the top left. All data are approximate.

A correlation between two variables indicates only that the variables are associated; it does not imply a cause-and-effect relation. Nor does correlation mean that the values of two variables are the same; it means only that a change in one variable is associated with a proportional change in the other variable. For example, the *x* and *y* variables in the following list are almost perfectly correlated, with a correlation coefficient of 0.99.

	***x* value**	***y* value**
	12	123
	14	140

	10	110
	6	61
	3	32
Average:	9	93

A strong correlation is found between these *x* and *y* variables; larger values of *x* are always associated with larger values of *y*. Note that the *y* values are about 10 times as large as the corresponding *x* values, so although *x* and *y* are correlated, they are not identical. The distinction between correlation and identity becomes important when we consider the effects of heredity and environment on the correlation of characteristics. **› TRY PROBLEM 26**

THINK-PAIR-SHARE Question 3

Regression

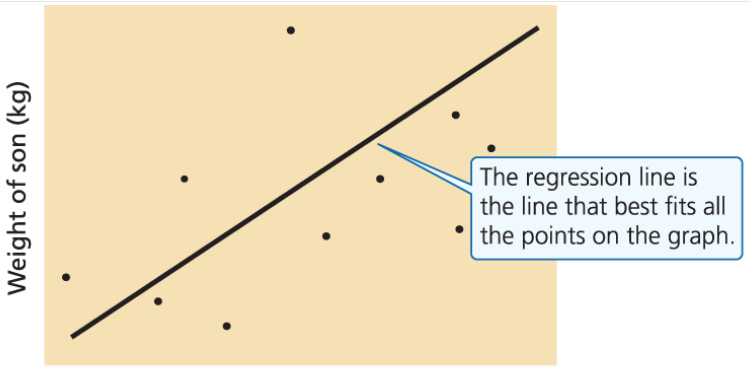
Correlation provides information only about the strength and direction of association between variables. However, we often want to know more than whether two variables are associated: we want to be able to predict the value of one variable given a value of the other.

A positive correlation exists between the body weight of parents and the body weight of their offspring; this correlation exists in part because genes influence body weight, and parents and offspring have genes in common. Because of this association between parental and offspring phenotypes, we can predict the weight of an individual on the basis of the weights of its parents. This type of statistical prediction is called **regression**. This technique plays an important role in quantitative genetics because it allows us to predict the characteristics of offspring of a given mating, even without knowledge of the genotypes that encode those characteristics.

Regression can be understood by plotting a series of *x* and *y* values. **Figure 24.13** illustrates the relation between the weight of a father (*x*) and the weight of his son (*y*). Each father–son pair is represented by a point on the graph. The overall relation between these two variables is depicted by the regression line, which is the line that best fits all the points on the graph (deviations of the points from the line are minimized). The regression line defines the relation between the *x* and *y* variables and can be represented by

$$y = a + bx$$

(24.8)



24.13 A regression line defines the relation between two variables. Illustrated here is a regression of the weights of fathers against the weights of sons. Each father–son pair is represented by a point on the graph: the *x* value of a point is the father's weight and the *y* value of the point is the son's weight.

Description
The horizontal axis represents weight of father (k g). The vertical axis represents weight of son (k g). A line with positive slope is present with multiple dots scattered throughout the plot. A caption reads, The regression line is the line that best fits all the points on the graph.

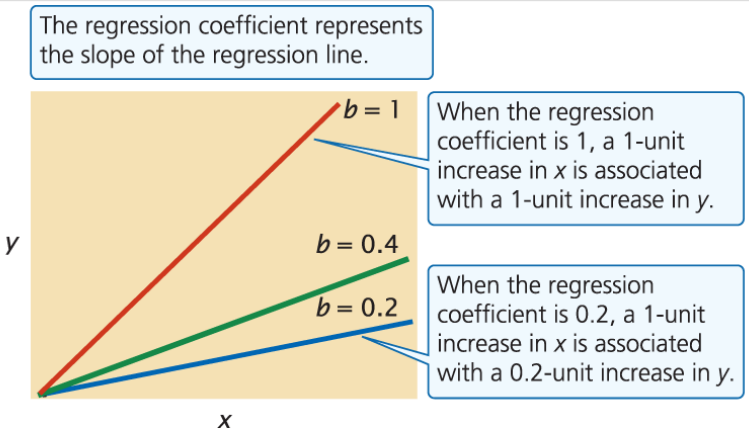
In Equation 24.8, x and y represent the x and y variables (in this case, the father's weight and the son's weight, respectively). The variable a is the y intercept of the regression line, which is the expected value of y when x is 0. The variable b is the slope of the regression line, also called the **regression coefficient**.

Positioning a regression line by eye is not only very difficult but unlikely to be accurate when there are many points scattered over a wide area. Fortunately, the regression coefficient and the y intercept can be obtained mathematically. The regression coefficient (b) can be computed from the covariance of x and y (cov_{xy}) and the variance of x (s_x^2) by

$$b = \frac{\text{cov}_{xy}}{s_x^2}$$

(24.9)

The regression coefficient indicates how much y increases, on average, per increase in x. Several regression lines with different regression coefficients are illustrated in Figure 24.14. Notice that as the regression coefficient increases, the slope of the regression line increases.



24.14 The regression coefficient, *b*, represents the change in *y* per unit change in *x*. Shown here are regression lines with different regression coefficients.

Description
The horizontal axis is labeled x and the vertical axis is labeled y. Text at the top reads, The regression coefficient represents the slope of the regression line. Three lines with positive slope are present. The top most line shows b equals 1. A caption reads, When the regression coefficient is 1, a 1-unit increase in x is associated with a 1-unit increase in y. A line at the center shows b equals 0 point 4. A line at the bottom shows b equals 0 point 2. A caption reads, When the regression coefficient is 0 point 2, a 1-unit increase in x is associated with a 0 point 2-unit increase in y.

After the regression coefficient has been calculated, the y intercept can be calculated by substituting the regression coefficient and the mean values of x and y into the following equation:

$$a = y - bx \tag{24.10}$$

The regression equation ($y = a + bx$; Equation 24.8) can then be used to predict the value of any y given the value of x.

CONCEPTS

A correlation coefficient measures the strength of association between two variables. The sign (positive or negative) indicates the direction of the correlation; the absolute value measures the strength of the association. Regression is used to predict the value of one variable on the basis of the value of a correlated variable.

✔ CONCEPT CHECK 4

In Lubbock, Texas, rainfall and temperature exhibit a significant correlation of −0.7. Which conclusion is correct?

a. There is usually more rainfall when the temperature is high.
b. There is usually more rainfall when the temperature is low.
c. Rainfall is equally likely when the temperature is high or low.

WORKED PROBLEM

The body weights of 11 female fish and the numbers of eggs they produce are given in the following table:

Weight (mg)	Eggs (thousands)
x	***y***
14	61
17	37
24	65
25	69
27	54
33	93
34	87
37	89
40	100
41	90
42	97

What are the correlation coefficient and the regression coefficient for body weight and egg number in these 11 fish?

Solution Strategy

What information is required in your answer to the problem?

The correlation coefficient (r) and the regression coefficient (b) for body weight and egg number in the fish.

What information is provided to solve the problem?

Body weights and egg numbers for a sample of 11 fish.

Solution Steps

The computations needed to answer this question are given in the table on the facing page. To calculate the correlation and regression coefficients, we first obtain the sum of all the x_i values ($\sum x_i$) and the sum of all the y_i values ($\sum y_i$); these sums are shown in the last row of the table. We can calculate the means of the two variables by dividing the sums by the number of measurements, which is 11:

$$x = \frac{\sum x_i}{n} = \frac{334}{11} = 30.36$$
$$y = \frac{\sum y_i}{n} = \frac{842}{11} = 76.55$$

A	B	C	D	E	F	G
Weight (mg)			**Eggs (thousands)**			
x	$x_i - x$	$(x_i - x)^2$	y	$y_i - y$	$(y_i - y)^2$	$(x_i - x)(y_i - y)$
14	−16.36	267.65	61	−15.55	241.80	254.40
17	−13.36	178.49	37	−39.55	1564.20	528.39
24	−6.36	40.45	65	−11.55	133.40	73.46
25	−5.36	28.73	69	−7.55	57.00	40.47
27	−3.36	11.29	54	−22.55	508.50	75.77
33	2.64	6.97	93	16.45	270.60	43.43
34	3.64	13.25	87	10.45	109.20	38.04
37	6.64	44.09	89	12.45	155.00	82.67

40	9.64	92.93	100	23.45	549.90	226.06
41	10.64	113.21	90	13.45	180.90	143.11
42	11.64	135.49	97	20.45	418.20	238.04
$\sum x_i = 334$		$\sum (x_i - x)^2 = 932.55$	$\sum y_i = 842$		$\sum (y_i - y)^2 = 4188.70$	$\sum (x_i - x)(y_i - y) = 1743.84$

Data from R. R. Sokal and F. J. Rohlf, *Biometry*, 2d ed. San Francisco: W. H. Freeman, 1981.

After the means have been calculated, the deviations of each value from the mean are computed; these deviations are shown in columns B and E of the table. The deviations are then squared (columns C and F) and summed (last row of columns C and F). Next, the products of the deviation of the x values and the deviation of the y values ($(x_i - x)(y_i - y)$) are calculated; these products are shown in column G, and their sum is shown in the last row of column G.

To calculate the covariance, we use Equation 24.6:

$$\text{cov}_{xy} = \frac{\sum (x_i - x)(y_i - y)}{n - 1} = \frac{1743.84}{10} = 174.38$$

Calculating the correlation and the regression requires the variances and standard deviations of x and y:

$$\begin{aligned} s_x^2 &= \frac{\sum (x_i - x)^2}{n - 1} = \frac{932.55}{10} = 93.26 \\ s_x &= \sqrt{s_x^2} = \sqrt{93.26} = 9.66 \\ s_y^2 &= \frac{\sum (y_i - y)^2}{n - 1} = \frac{4188.70}{10} = 418.87 \\ s_y &= \sqrt{s_y^2} = \sqrt{418.87} = 20.47 \end{aligned}$$

We can now compute the correlation and regression coefficients as shown here.

Correlation coefficient:

$$r = \frac{\text{cov}_{xy}}{s_x s_y} = \frac{174.38}{9.66 \times 20.47} = 0.88$$

Regression coefficient:

$$b = \frac{\text{cov}_{xy}}{s_x^2} = \frac{174.38}{93.26} = 1.87$$

» Practice your understanding of correlation and regression by working **Problem 27** at the end of the chapter.

Applying Statistics to the Study of a Polygenic Characteristic

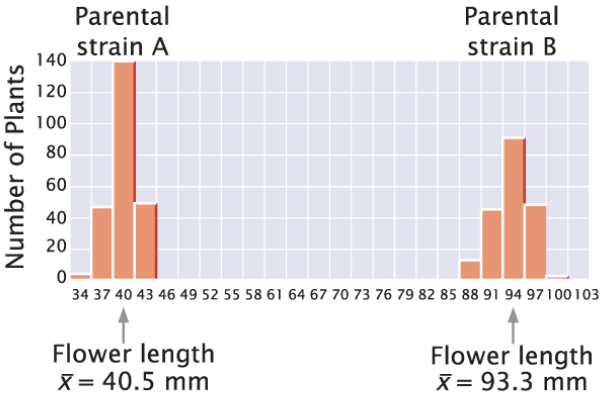
Edward East carried out an early statistical study of polygenic inheritance on the length of flowers in tobacco (*Nicotiana longiflora*). He obtained two varieties of tobacco that differed in flower length: one variety had a mean flower length of 40.5 mm, and the other had a mean flower length of 93.3 mm (**Figure 24.15**). Each of these two varieties had been inbred for many generations and was homozygous at all loci contributing to flower length. Thus, there was no genetic variation in the original parental strains; the small variations in flower length within each strain were due to environmental effects on flower length.

Experiment

Question: How is flower length in tobacco plants inherited?

Methods

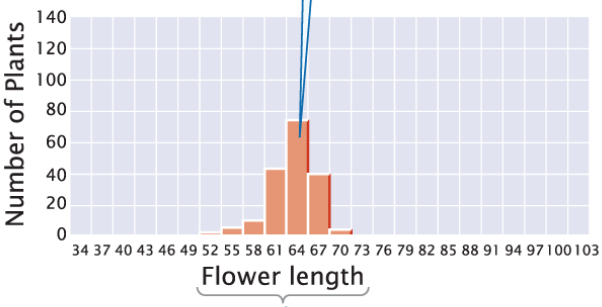
P generation



Results

F_1 generation

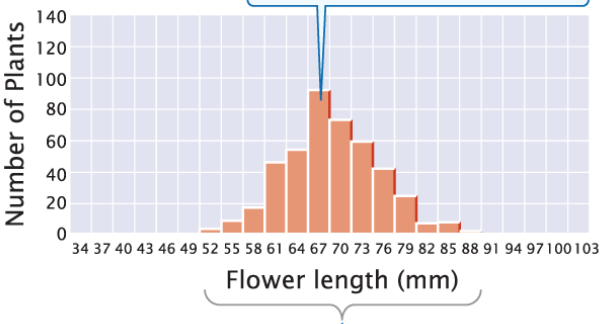
1 Flower length in the F_1 was about halfway between that in the two parents,…



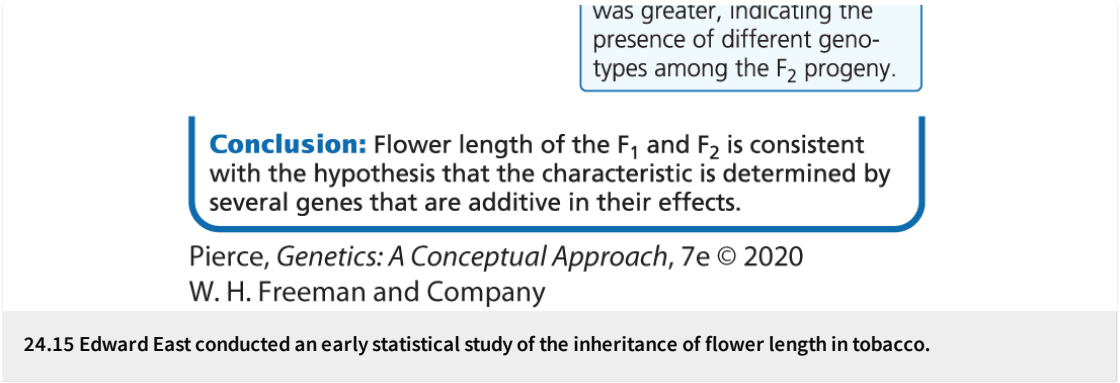
2 …and the variance in the F_1 was similar to that seen in the parents.

F_2 generation

3 The mean of the F_2 was similar to that observed for the F_1,…



4 …but the variance in the F_2



24.15 Edward East conducted an early statistical study of the inheritance of flower length in tobacco.

Description

The illustration shows a question that reads, How is flower length in tobacco plants inherited?

A white flower is shown below and its center part is labeled as flower length. The Methods section below shows P generation.

Three bar graphs below shows number of plants plotted against flower length. The horizontal axis has the following points: 34 to 103, in increments of 3. The vertical axis represents Number of Plants and ranges from 0 to 140, in increments of 20.

In the first graph, for parental strain A, four bars extend up to (34, 2), (37, 43), (40, 140), (43, 45). Text below bar that extends up to (40, 140) reads, flower length x bar equals 40 point 5 m m. For parental strain A, five bars extend up to (88, 15), (91, 43), (94, 90), (97, 43), (100, 1).). Text below bar that extends up to (94, 90) reads, flower length x bar equals 93 point 3 m m. All data are approximate.

The results section below shows F subscript 1 generation.

In the second graph, seven bars extend up to (52, 2), (55, 3), (58, 10), (61, 42), (64, 76), (67, 40), and (70, 5). A caption reads, Flower length in the F subscript 1 was about halfway between that in the two parents, and the variance in the F subscript 1 was similar to that seen in the parents. All data are approximate.

F subscript 2 generation below shows the third bar graph. Twelve bars extend up to (52, 2), (55, 8), (58, 18), (61, 42), (64, 56), (67, 91), (70, 74), (73, 60), (76, 41), (79, 25), (82, 7) and (85, 8). A caption reads, The mean of the F subscript 2 was similar to that observed for the F subscript 1 but the variance in the F subscript 2 was greater, indicating the presence of different genotypes among the F subscript 2 progeny. All data are approximate.

A caption below shows, Conclusion: Flower length of the F subscript 1 and F subscript 2 is consistent with the hypothesis that the characteristic is determined by several genes that are additive in their effects.

When East crossed the two strains, he found that the mean flower length of the F_1 was about halfway between the mean flower lengths of the two parents (see Figure 24.15), as would be expected if the genes determining the difference between the two strains were additive in their effects. The variance of flower length in the F_1 was similar to that seen in the parental strains, because all the F_1 had the same genotype, as did each parental strain (the F_1 were all heterozygous at the genes that differed between the two parental varieties).

East then interbred the F_1 to produce F_2 progeny. The mean flower length of the F_2 was similar to that of the F_1, but the variance of the F_2 was much greater (see Figure 24.15). This greater

variation indicates that not all of the F_2 progeny had the same genotype.

East selected some F_2 plants and interbred them to produce F_3 progeny. He found that flower length in the F_3 depended on flower length in the plants selected as their parents. This finding demonstrated that flower-length differences in the F_2 were partly genetic and were therefore passed to the next generation. None of the 444 F_2 plants raised by East exhibited flower lengths similar to those of the two parental strains. This result suggested that more than four loci with pairs of alleles affected flower length in his varieties because four allelic pairs are expected to produce 1 of 256 progeny $[(1/4)^4 = 1/256]$ having one or the other of the original parental phenotypes.

24.3 Heritability Is Used to Estimate the Proportion of Variation in a Trait That Is Genetic

In addition to being polygenic, quantitative characteristics are frequently influenced by environmental factors. Knowing how much of the variation in a quantitative characteristic is due to genetic differences and how much is due to environmental differences is often useful. The proportion of the total phenotypic variation that is due to genetic differences is known as **heritability**.

Consider a dairy farmer who owns several hundred milk cows. The farmer notices that some cows consistently produce more milk than others. The nature of these differences is important to the profitability of the farmer's dairy operation. If the differences in milk production are largely genetic in origin, then the farmer may be able to boost milk production by selectively breeding the cows that produce the most milk. On the other hand, if the differences are largely environmental in origin, selective breeding will have little effect, and the farmer might better boost milk production by adjusting the environmental factors associated with higher milk production. To determine the extent of genetic and environmental influences on variation in a characteristic, phenotypic variation in that characteristic must be partitioned into components attributable to different factors.

Phenotypic Variance

To determine how much of the phenotypic variation in a population is due to genetic factors and how much is due to environmental factors, we must first have some quantitative measure of the phenotype under consideration. Consider a population of wild plants that differ in size. We could collect a representative sample of plants from the population, weigh each plant in the sample, and calculate the mean and variance of plant weight. This **phenotypic variance** is represented by V_P.

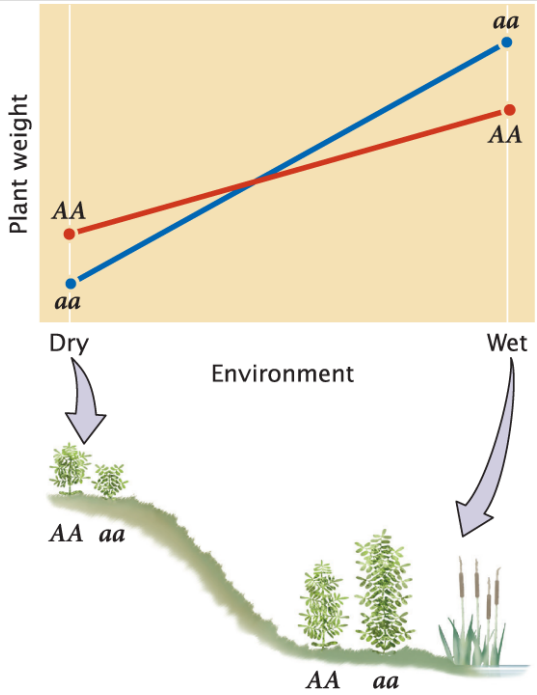
COMPONENTS OF PHENOTYPIC VARIANCE

Phenotypic variance can be divided into several components. First, some of the phenotypic variance may be due to differences in genotypes among individual members of the population. These differences are termed the **genetic variance** and are represented by V_G.

Second, some of the differences in phenotype may be due to environmental differences among the plants; these differences are termed the **environmental variance**, V_E. Environmental variance includes differences that result from environmental factors such as the amount of light or water that the plant receives; it also includes random differences in development that cannot be attributed to any

specific factor. Any variation in phenotype that is not inherited is, by definition, a part of the environmental variance.

Third, **genetic–environmental interaction variance** (V_{GE}) arises when the effect of a gene depends on the specific environment in which it is found. An example is shown in **Figure 24.16**. In a dry environment, genotype *AA* produces a plant that averages 12 g in weight, and genotype *aa* produces a smaller plant that averages 10 g. In a wet environment, genotype *aa* produces the larger plant, averaging 24 g in weight, whereas genotype *AA* produces a plant that averages 20 g. In this example, there are clearly differences in the two environments: both genotypes produce heavier plants in the wet environment. There are also differences in the weights of the two genotypes, but the relative performances of the genotypes depend on whether the plants grow in a wet or a dry environment. In this case, the influences on phenotype cannot be neatly allocated to genetic and environmental components because the expression of the genotype depends on the environment in which the plant grows. The phenotypic variance must therefore include a component that accounts for the way in which genetic and environmental factors interact.



Pierce, *Genetics: A Conceptual Approach*, 7e
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24.16 Genetic–environmental interaction variance arises when the effect of a gene depends on the specific environment in which the organism is found. In this theoretical example, the genotype affects plant weight, but the environmental conditions determine which genotype produces the heavier plant.

Description

The line graph shows environment on horizontal axis and plant weight on vertical axis. The horizontal axis shows dry on the left side and wet on the right side. A positive blue line representing small A small A starts a little above origin on left side of horizontal axis indicating dry and reaches to the top right side of the horizontal axis over wet. A positive red line representing big A big A starts above the blue line over dry and ends below the blue line on the right side, over wet. A dot of respective colors is present on each side of the line, for both the lines.

The illustration below shows 2 plants at the top of a hill in a dry area and 2 plants at the bottom of the hill near a pond. A tall plant with genotype big A big A and a small plant with genotype small A small A are present at the top of the hill corresponding to the dry area. A small plant with genotype big A big A and a tall plant has genotype small A small A are present at the bottom of the hill corresponding to the wet area. An arrow from dry area and

another arrow from wet area, marked at the horizontal axis above points to the corresponding plants below.

In summary, the total phenotypic variance can be apportioned into three components:

$$V_{\mathrm{P}} = V_{\mathrm{G}} + V_{\mathrm{E}} + V_{\mathrm{GE}} \tag{24.11}$$

COMPONENTS OF GENETIC VARIANCE

Genetic variance can be further subdivided into components consisting of different types of genetic effects. First, **additive genetic variance** (V_{A}) comprises the additive effects of genes on the phenotype, which can be summed to determine the overall effect on the phenotype (see Section 24.1). For example, suppose that in a plant, allele A^1 contributes 2 g in weight and allele A^2 contributes 4 g. If the effects of the alleles are strictly additive, then the genotypes would have the following weights:

$$\begin{aligned} A^1A^1 &= 2 + 2 = 4\,\mathrm{g} \\ A^1A^2 &= 2 + 4 = 6\,g \\ A^2A^2 &= 4 + 4 = 8\,\mathrm{g} \end{aligned}$$

The genes studied by Nilsson-Ehle that affect kernel color in wheat were additive in this way. It is the additive genetic variance that primarily determines the resemblance between parents and

offspring. For example, if all of the phenotypic variance is due to additive genetic variance, then the average phenotype of the offspring will be exactly intermediate between the phenotypes of the parents.

Second, there is **dominance genetic variance** (V_D) when some genes have a dominance component. In this case, the alleles at a locus are not additive; rather, the effect of an allele depends on the identity of the other allele at that locus. For example, with a dominant allele (*T*), genotypes *TT* and *Tt* have the same phenotype. Here, we cannot simply add the effects of the alleles together because the effect of the *t* allele is masked by the presence of the *T* allele. Instead, we must add a component (V_D) to the genetic variance to account for the way in which alleles interact.

Third, genes at different loci may interact in the same way that alleles at the same locus interact. When this gene interaction takes place, the effects of the genes are not additive. Coat color in Labrador retrievers, for example, exhibits gene interaction, as described in Section 5.2: genotypes *BB ee* and *bb ee* both produce yellow dogs because the effects of alleles at the *B* locus are masked when two *e* alleles are present at the *E* locus. With gene interaction, we must add a third component, called **gene interaction variance** (V_I), to the genetic variance:

$$V_G = V_A + V_D + V_I$$

(24.12)

SUMMARY EQUATION

We can now integrate these components into one equation to represent all the potential contributions to the phenotypic variance:

$$V_{\mathrm{P}} = V_{\mathrm{A}} + V_{\mathrm{D}} + V_{\mathrm{I}} + V_{\mathrm{E}} + V_{\mathrm{GE}}$$

(24.13)

This equation provides us with a model that describes the potential causes of differences we observe among individual phenotypes. It's important to note that this model deals strictly with observable *differences* (variance) in phenotypes among individual members of a population. It says nothing about the absolute values of characteristics or about the underlying genotypes that produce these differences.

Types of Heritability

The model of phenotypic variance that we've just developed can be used to determine how much of the phenotypic variance in a characteristic is due to genetic differences. **Broad-sense heritability** (H^2) represents the proportion of phenotypic variance that is due to genetic variance. It is calculated by dividing the genetic variance by the phenotypic variance:

$$\text{broad-sense heritability} = H^2 = \frac{V_\text{G}}{V_\text{P}}$$

(24.14)

The symbol H^2 represents broad-sense heritability because it is a measure of variance, which is given in units squared.

Broad-sense heritability can potentially range from 0 to 1. A value of 0 indicates that none of the phenotypic variance results from differences in genotype and all of the differences in phenotype result from environmental variation. A value of 1 indicates that all of the phenotypic variance results from differences in genotype. A heritability value between 0 and 1 indicates that both genetic and environmental factors influence the phenotypic variance.

Often, geneticists are more interested in the proportion of the phenotypic variance that results from the additive genetic variance because, as mentioned earlier, the additive genetic variance primarily determines the resemblance between parents and offspring. **Narrow-sense heritability** (h^2) is equal to the additive genetic variance divided by the phenotypic variance:

$$\text{narrow-sense heritability} = h^2 = \frac{V_\text{A}}{V_\text{P}}$$

(24.15)

› **TRY PROBLEM 28**

Calculating Heritability

Now that we have considered the components that contribute to phenotypic variance and developed a general concept of heritability, we can ask how to go about estimating these different components and calculating heritability. There are several ways to measure the heritability of a characteristic. They include eliminating one or more variance components, comparing the resemblance of parents and offspring, comparing the phenotypic variances of individuals with different degrees of relatedness, and measuring the response to selection (this last method will be discussed in Section 24.4). The mathematical theory that underlies these calculations of heritability is complex, so we focus here on developing a general understanding of how heritability is measured.

HERITABILITY BY ELIMINATION OF VARIANCE COMPONENTS

One way of calculating broad-sense heritability is to eliminate one of the components of phenotypic variance. We have seen that $V_P = V_G + V_E + V_{GE}$. If we eliminate all environmental variance ($V_E = 0$), then $V_{GE} = 0$ (because if either V_G or V_E equals 0, no genetic–environmental interaction can take place), and $V_P = V_G$. In theory, we might make V_E equal to 0 by ensuring that all individuals are raised in exactly the same environment, but in practice, it is virtually impossible. Instead, we could make V_G equal to 0 by raising

genetically identical individuals, causing V_{P} to be equal to V_{E}. In a typical experiment, we might raise cloned or highly inbred, identically homozygous individuals in a defined environment and measure their phenotypic variance to estimate V_{E}. We might then raise a group of genetically variable individuals and measure their phenotypic variance (V_{P}). Using the V_{E} calculated for the genetically identical individuals, we could obtain the genetic variance of the variable individuals by subtraction:

$$V_{\mathrm{G\ (of\ genetically\ varying\ individuals)}} = V_{\mathrm{P\ (of\ genetically\ varying\ individuals)}} - V_{\mathrm{E\ (of\ genet}$$

(24.16)

The broad-sense heritability of the genetically variable individuals would then be calculated as follows:

$$H^2 = \frac{V_{\mathrm{G\ (of\ genetically\ varying\ individuals)}}}{V_{\mathrm{P\ (of\ genetically\ varying\ individuals)}}}$$

(24.17)

Sewall Wright used this method to estimate the heritability of white spotting in guinea pigs. He first measured the phenotypic variance of white spotting in a genetically variable population and found that $V_{\mathrm{P}} = 573$. Then he inbred the guinea pigs for many generations so that they were essentially homozygous and genetically identical. When he measured the phenotypic variance of white spotting in the inbred group, he obtained $V_{\mathrm{P}} = 340$. Because $V_{\mathrm{G}} = 0$ in this group,

their $V_P = V_E$. Wright assumed this value of environmental variance for the original (genetically variable) population and estimated their genetic variance:

$$V_P - V_E = V_G$$
$$573 - 340 = 233$$

He then estimated broad-sense heritability from the genetic and phenotypic variance:

$$H^2 = \frac{V_G}{V_P}$$
$$H^2 = \frac{233}{573} = 0.41$$

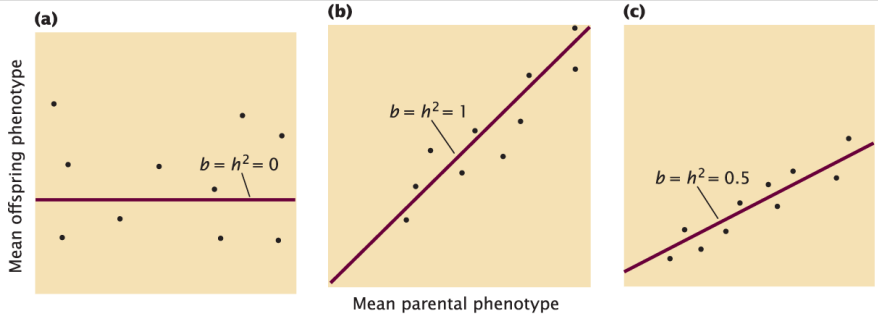
This value implies that 41% of the variation in white spotting in guinea pigs in Wright's population was due to differences in genotype.

Estimating heritability with this method assumes that the environmental variance of genetically identical individuals is the same as the environmental variance of genetically variable individuals, which may not be true. Additionally, this approach can be applied only to organisms for which it is possible to create genetically identical individuals. › **TRY** PROBLEM 33

HERITABILITY BY PARENT–OFFSPRING REGRESSION

Another method of estimating heritability is to compare the phenotypes of parents with those of their offspring. When genetic differences are responsible for phenotypic variance, offspring should resemble their parents more than they resemble unrelated individuals because offspring and parents share some genes that help determine their phenotype. Correlation and regression can be used to analyze the association of phenotypes in different individuals.

To calculate the narrow-sense heritability of a characteristic in this way, we first measure the characteristic in a series of parents and offspring. The data are arranged into families, and the mean parental phenotype is plotted against the mean offspring phenotype (Figure 24.17). Each data point in the graph represents one family; the value on the x (horizontal) axis is the mean phenotypic value of the parents in a family, and the value on the y (vertical) axis is the mean phenotypic value of the offspring in the family.



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24.17 Narrow-sense heritability, h^2, equals the regression coefficient, b, in a regression of the mean phenotype of offspring against the mean phenotype of their parents. (a) There is no relation between the mean parental phenotype and the mean offspring phenotype. (b) The mean offspring phenotype is the same as

the mean parental phenotype. (c) Both genes and environment contribute to phenotypic differences.

Description
All the horizontal axes represent mean parental phenotype and all the vertical axes represent mean offspring phenotype.

Scatter plot a shows a horizontal line just above the horizontal axis. The line is labeled b equals h squared equals 0. The dots are spread throughout the graph.

Scatter plot b shows a line with positive slope. The line is labeled b equals h squared equals 1, begins from origin extends till the top right corner of the plot. The dots are spread close to the line.

Scatter plot c shows a line with a shallow positive slope. The line is labeled b equals h squared equals 0 point 5, begins a little above origin and ends at the center right of the plot. The dots are very close to the line.

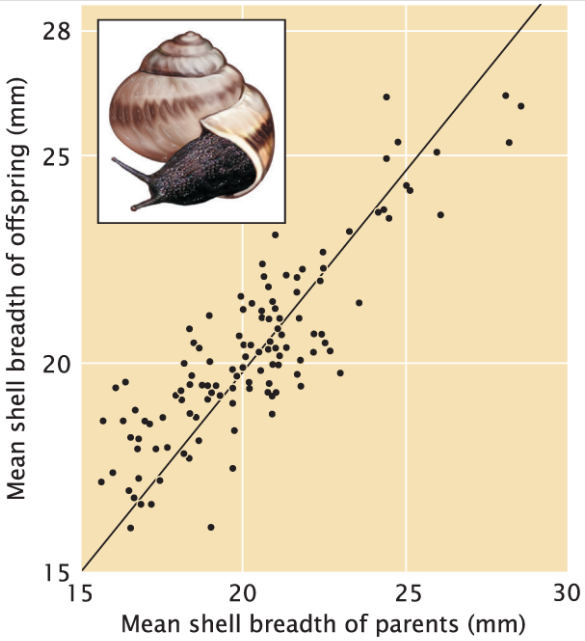
Let's begin by assuming that there is no narrow-sense heritability for the characteristic ($h^2 = 0$), meaning that genetic differences do not contribute to the phenotypic differences among individuals. In this case, offspring will be no more similar to their parents than they are to unrelated individuals, and the data points will be scattered randomly, generating a regression coefficient of 0 (see Figure 24.17a). Next, let's assume that all of the phenotypic differences are due to additive genetic differences ($h^2 = 1$). In this case, the mean phenotype of the offspring will be equal to the mean phenotype of the parents, and the regression coefficient will be 1 (see Figure 24.17b). Finally, if genes and environment both contribute to the phenotypic differences, both heritability and the regression coefficient will lie between 0 and 1 (see Figure 24.17c). The

regression coefficient therefore provides information about the magnitude of heritability.

A complex mathematical proof (which we will not go into here) demonstrates that in a regression of the mean phenotype of the offspring against the mean phenotype of the parents, narrow-sense heritability (h^2) equals the regression coefficient (b):

$$h^2 = b_{\text{(regression of offspring mean against mean of both parents)}} \tag{24.18}$$

An example of calculating heritability by regression of the phenotypes of parents and offspring is illustrated in **Figure 24.18**.



24.18 The heritability of shell breadth in snails can be determined by regression of the mean phenotype of offspring against the mean phenotype of their

parents. The regression coefficient, which equals the heritability, is 0.70. [Data from L. M. Cook, *Evolution* 19:86–94, 1965.]

Description
The horizontal axis represents Mean shell breadth of parents (m m) and ranges from 15 to 30, in increments of 5. The vertical axis represents Mean shell breadth of offspring (m m) and ranges from 15 to 28, in increments of 5. A line with positive slope starts from (15, 15) and ends at (29, 28 point 5). A large number of dots appear along the bottom half of the line, with fewer towards the top. A photo shows a snail at the top left. All data are approximate.

Sometimes only the phenotype of one parent is known. In a regression of the mean offspring phenotype against the phenotype of only one parent, the narrow-sense heritability equals twice the regression coefficient:

$$h^2 = 2b_{\text{(regression of offspring mean against mean of one parent)}} \tag{24.19}$$

With only one parent, the heritability is twice the regression coefficient because only half the genes of the offspring come from that one parent; thus, we must double the regression coefficient to obtain the full heritability.

HERITABILITY AND DEGREES OF RELATEDNESS

A third method for calculating heritability is to compare the phenotypes of individuals with different degrees of relatedness. This

method is based on the concept that the more closely related two individuals are, the more genes they share.

Monozygotic (identical) twins share 100% of their genes, whereas dizygotic (nonidentical) twins share, on average, 50% of their genes (see Section 6.3). If genes are important in determining variation in a characteristic, then monozygotic twins should be more similar in that characteristic than dizygotic twins. By using correlation to compare the phenotypes of monozygotic twins and of dizygotic twins, we can estimate broad-sense heritability. A rough estimate of broad-sense heritability can be obtained by taking twice the difference between the correlation coefficients for a quantitative characteristic in monozygotic and dizygotic twins:

$$H^2 = 2(r_{\mathrm{MZ}} - r_{\mathrm{DZ}}) \tag{24.20}$$

where r_{MZ} equals the correlation coefficient among monozygotic twins and r_{DZ} equals the correlation coefficient among dizygotic twins. For example, suppose we found the correlation of height among the two members of monozygotic twin pairs (r_{MZ}) to be 0.9 and the correlation of height among the two members of dizygotic twin pairs (r_{DZ}) to be 0.5. The broad-sense heritability for height would be $H^2 = 2(0.9 - 0.5) = 2(0.4) = 0.8$. This calculation assumes that the two members of a monozygotic twin pair experience environments that are no more similar to each other than those

experienced by the two members of a dizygotic twin pair. This assumption is often not met when twins have been reared together.

Narrow-sense heritability can be estimated by comparing the phenotypic variances for a characteristic in full siblings (who have both parents in common as well as an average of 50% of their genes) and in half siblings (who have only one parent in common and thus an average of 25% of their genes).

All these estimates of heritability depend on the assumption that the environments of related individuals are not more similar than those of unrelated individuals. This assumption is difficult to meet in human studies because related people are usually reared together. Therefore, heritability estimates for humans should always be viewed with caution. › **TRY** PROBLEM 37

CONCEPTS

Broad-sense heritability is the proportion of phenotypic variance that is due to genetic variance. Narrow-sense heritability is the proportion of phenotypic variance that is due to additive genetic variance. Heritability can be measured by eliminating one of the variance components, by analyzing parent–offspring regression, or by comparing groups of individuals with different degrees of relatedness.

✔ CONCEPT CHECK 5

If the environmental variance (V_E) increases and all other variance components remain the same, what will the effect be?

a. Broad-sense heritability will decrease.

b. Broad-sense heritability will increase.
c. Narrow-sense heritability will increase.
d. Broad-sense heritability will increase, but narrow-sense heritability will decrease.

The Limitations of Heritability

Knowledge of heritability has great practical value because it allows us to statistically predict the phenotypes of offspring on the basis of their parents' phenotypes. It also provides useful information about how characteristics will respond to selection (see Section 24.4). In spite of its importance, heritability is frequently misunderstood. It does not provide information about an individual's genes or the environmental factors that control the development of a characteristic, and it says nothing about the nature of differences between groups. This section outlines some limitations and common misconceptions of broad- and narrow-sense heritability.

HERITABILITY DOES NOT INDICATE THE DEGREE TO WHICH A CHARACTERISTIC IS GENETICALLY DETERMINED

Heritability is the proportion of the phenotypic variance that is due to genetic variance; it says nothing about the degree to which genes determine a characteristic. Heritability indicates only the degree to which genes determine *variation* in a characteristic. The

determination of a characteristic and the determination of variation in a characteristic are two very different things.

Consider polydactyly (the presence of extra digits) in rabbits, which can be caused either by environmental factors or by a dominant gene. Suppose that we have a group of rabbits that are all homozygous for a gene that produces the usual numbers of digits. None of the rabbits in this group carries a gene for polydactyly, but a few of the rabbits are polydactylous because of environmental factors. Broad-sense heritability for polydactyly in this group is zero because there is no genetic variation for polydactyly; all the variation is due to environmental factors. However, it would be incorrect for us to conclude that genes play no role in determining the number of digits in rabbits. Indeed, we know that there are specific alleles that can produce extra digits (although these alleles are not present in the group of rabbits under consideration). Heritability indicates nothing about whether genes control the development of a characteristic. It provides information only about causes of the variation in a characteristic within a defined group.

AN INDIVIDUAL DOES NOT HAVE HERITABILITY

Broad- and narrow-sense heritabilities are statistical values based on the genetic and phenotypic variances found in a *group* of individuals. Heritability cannot be calculated for an individual, and heritability has no meaning for a specific individual. Suppose that we calculate

the narrow-sense heritability of adult body weight for the students in a biology class and obtain a value of 0.6. We could conclude that 60% of the variation in adult body weight among the students in this class is determined by additive genetic variance. We should not, however, conclude that 60% of any particular student's body weight is due to additive genes.

THERE IS NO UNIVERSAL HERITABILITY FOR A CHARACTERISTIC

The heritability value for a characteristic is specific to a given population in a given environment. Recall that broad-sense heritability is genetic variance divided by phenotypic variance. Genetic variance depends on which alleles are present, which often differs between populations. In the example of polydactyly in rabbits, there were no alleles for polydactyly in the group of rabbits we considered, so the heritability of the characteristic was zero. A different group of rabbits might contain many alleles for polydactyly, and the heritability of the characteristic might be high in that group.

Environmental differences can also affect heritability because V_P is composed of both genetic and environmental variance. When the environmental factors that affect a characteristic differ between two groups, the heritabilities for the two groups often differ as well.

Because heritability is specific to a defined population in a given environment, it is important not to extrapolate heritabilities from one population to another. For example, human height is determined by environmental factors (such as nutrition and health) and by genes. If we measured the heritability of height in a developed country, we might obtain a value of 0.8, indicating that the variation in height in this population is largely genetic. Height has a high heritability in this population because most people have adequate nutrition and health care (V_E is low), so most of the phenotypic variation in height is genetically determined. It would be incorrect for us to assume, however, that height has a high heritability in all human populations. In developing countries, there may be more variation in a range of environmental factors; some people may enjoy good nutrition and health, whereas others may have a diet deficient in protein and suffer from diseases that affect stature. If we measured the heritability of height in such a country, we would undoubtedly obtain a lower value than we observed in the developed country because there would be more environmental variation, so the genetic variance in height would constitute a smaller proportion of the phenotypic variation, making the heritability lower. The important point to remember is that heritability must be calculated separately for each population and each environment.

EVEN WHEN HERITABILITY IS HIGH, ENVIRONMENTAL FACTORS CAN INFLUENCE A

CHARACTERISTIC

A high heritability value does not mean that environmental factors cannot influence the expression of a characteristic. High heritability indicates only that the environmental variation to which the population is *currently* exposed is not responsible for variation in the characteristic. Let's look again at human height. In most developed countries, the heritability of human height is high, indicating that genetic differences are responsible for most of the variation in height. It would be wrong for us to conclude, however, that human height cannot be changed by alterations in the environment. Indeed, height decreased in several European cities during World War II owing to hunger and disease, and height can be increased dramatically by the administration of growth hormone to children. The absence of environmental variation in a characteristic does not mean that the characteristic will not respond to environmental change.

HERITABILITIES INDICATE NOTHING ABOUT DIFFERENCES AMONG POPULATIONS

A common misconception about heritability is that it provides information about the nature of population differences in a characteristic. Heritability is specific to a given population in a given environment, so it cannot be used to draw conclusions about why populations differ in a characteristic.

Suppose that we measure the heritability of human height in two groups. One group is from a small town in a developed country, where everyone consumes a high-protein diet. Because there is little variation in the environmental factors that affect human height in this group and because there is some genetic variation, the heritability of height in this group is high. The second group comprises the inhabitants of a single village in a developing country. These people consume only 25% as much protein as those in the first group, so their average adult height is several centimeters less than that of the group from the developed country. Again, there is little variation in the environmental factors that determine height in this group because everyone in the village eats the same types of food and is exposed to the same diseases. Because there is little environmental variation and there is some genetic variation, the heritability of height in this group is also high.

Thus, the heritability of height in both groups is high, and the average height differs considerably between the two groups. We might be tempted to conclude that the difference in height between the two groups is genetically based—that the people in the developed country are genetically taller than the people in the developing country. This conclusion is obviously wrong, however, because these differences in height are due largely to diet, an environmental factor. Heritability provides no information about the causes of differences between populations.

These limitations of heritability have often been ignored, particularly in arguments about the possible social implications of genetic differences among humans. Soon after Mendel's principles of heredity were rediscovered, some geneticists began to claim that many human behavioral characteristics are determined entirely by genes. This claim led to debates about whether characteristics such as human intelligence are determined by genes or environment. Many of the early claims of genetically based human behavior were based on poor research; unfortunately, the results of these studies were often accepted at face value and led to a number of eugenic laws that discriminated against certain groups of people (see Section 25.4). Today, geneticists recognize that many behavioral characteristics are influenced by a complex interaction of genes and environment, and that separating genetic effects from those of the environment is very difficult.

The results of a number of modern studies indicate that human intelligence, as measured by IQ and other intelligence tests, has a moderately high heritability (usually from 0.4 to 0.8). On the basis of this observation, some people have argued that intelligence is innate and that enhanced educational opportunities cannot boost intelligence. This argument is based on the misconception that when heritability is high, changing the environment will not alter the characteristic. In addition, because heritability values for intelligence range from 0.4 to 0.8, a considerable amount of the variance in intelligence originates from environmental differences.

Another argument based on a misconception about heritability is that ethnic differences in measures of intelligence are genetically based. Because the results of some genetic studies show that IQ has moderately high heritability and because other studies find differences in average IQ among ethnic groups, some people have suggested that ethnic differences in IQ are genetically based. As in the example of the effects of diet on human height, heritability provides no information about causes of differences among groups; it indicates only the degree to which phenotypic variance within a single group is genetically based. High heritability for a characteristic does not mean that phenotypic differences in that characteristic among ethnic groups are genetic. We should also remember that because separating genetic and environmental effects in humans is so difficult, heritability estimates themselves may be unreliable. The limitations of heritability are summarized in Table 24.2. ❯ **TRY** PROBLEM 36

THINK-PAIR-SHARE Questions 4 and 5

TABLE 24.2 Limitations of heritability

1. Heritability does not indicate the degree to which a characteristic is genetically determined.
2. An individual does not have heritability.
3. There is no universal heritability for a characteristic.

4. Even when heritability is high, environmental factors can influence a characteristic.

5. Heritabilities indicate nothing about the nature of population differences in a characteristic.

CONCEPTS

Heritability provides information only about the degree to which *variation* in a characteristic is genetically determined. There is no universal heritability for a characteristic; heritability is specific to a given population in a specific environment. Environmental factors can potentially affect characteristics with high heritability, and heritability says nothing about the nature of differences among populations in a characteristic.

✔ CONCEPT CHECK 6

Suppose that you just learned that the narrow-sense heritability of blood pressure measured among a group of African Americans in Detroit, Michigan, is 0.4. What does this heritability tell you about genetic and environmental contributions to blood pressure?

Locating Genes That Affect Quantitative Characteristics

The statistical methods we have just described can be used both to make predictions about the average phenotype expected in offspring and to estimate the overall contribution of genes to variation in a

characteristic. These methods do not, however, allow us to identify and determine the influence of individual genes that affect quantitative characteristics. As discussed in the introduction to this chapter, chromosomal regions containing genes that control polygenic characteristics are referred to as **quantitative trait loci (QTLs)**. Although quantitative genetics has made important contributions to basic biology and to plant and animal breeding, the past inability to identify QTLs and measure their individual effects severely limited the application of quantitative genetic methods.

MAPPING QTLs

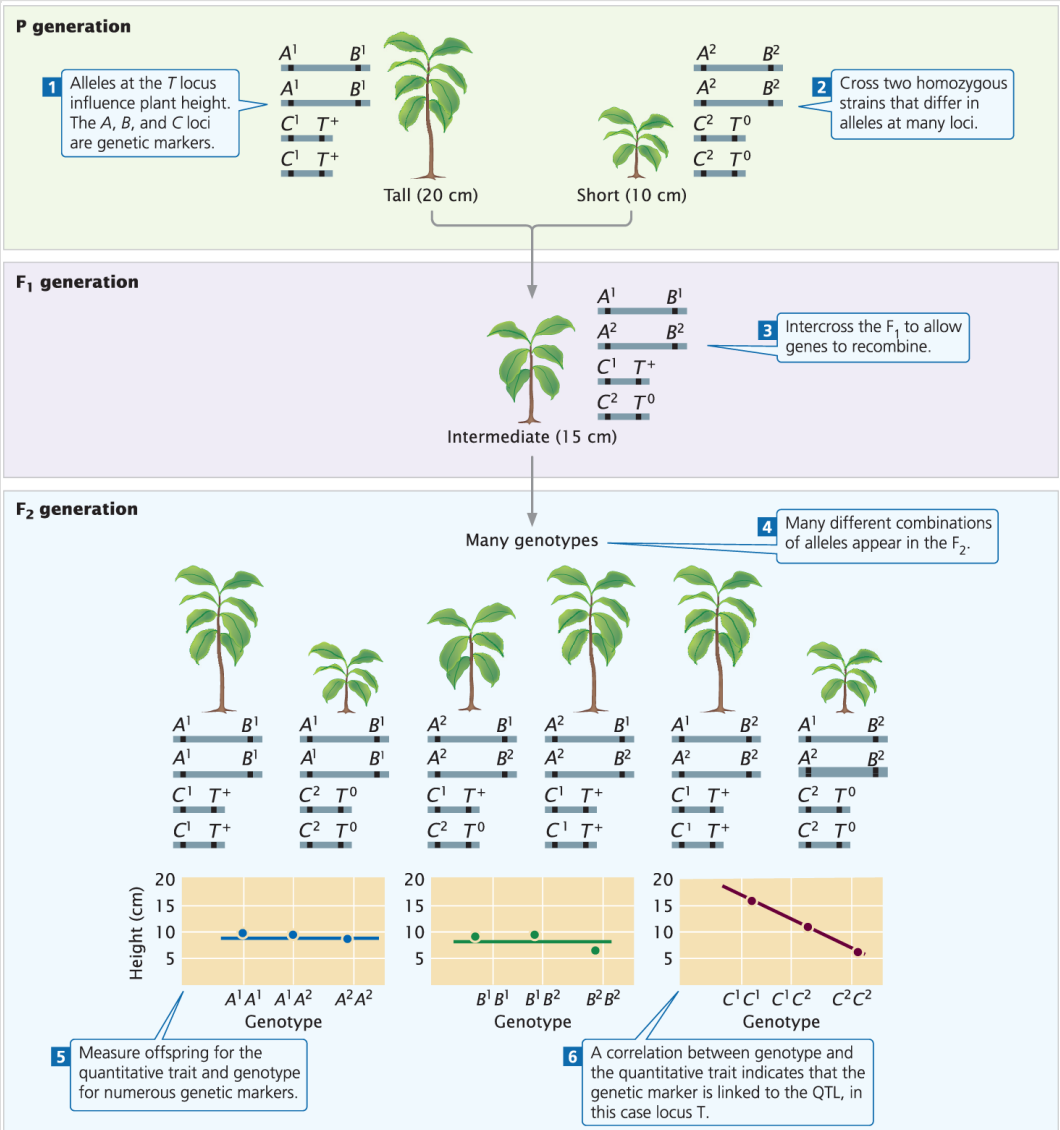
In recent years, numerous genetic markers have been identified and mapped with the use of molecular techniques, making it possible to identify QTLs by linkage analysis. The underlying idea is simple: if the inheritance of a genetic marker is associated consistently with the inheritance of a particular phenotype, such as increased height, then that marker must be linked to a QTL that affects height. The key is to have enough genetic markers so that QTLs can be detected throughout the genome. With the introduction of microsatellites and single-nucleotide polymorphisms (see Sections 19.5 and 20.1), variable markers are now available for mapping QTLs in a number of different organisms (**Figure 24.19**).

USDA.

24.19 QTL mapping is used to identify genes that influence many important quantitative traits, including muscle mass in pigs.

A common procedure for mapping QTLs is to cross two homozygous strains that differ in alleles at many loci (Figure 24.20). The resulting F_1 progeny are then intercrossed or backcrossed to allow the genes to recombine through independent assortment and crossing over. Genes on different chromosomes and genes that are far apart on the same chromosome will recombine freely; genes that are closely linked will be inherited together. The F_2 progeny are measured for one or more quantitative characteristics; at the same time, they are genotyped for numerous genetic markers that span the genome. Any correlation between the inheritance of a particular marker allele and that of a quantitative phenotype suggests that a QTL is physically linked to that marker. If enough markers are used, the detection of all the QTLs affecting a characteristic is theoretically possible. It is important to recognize that a QTL is not a gene; rather, it is a map location for a chromosomal region that is associated with a quantitative trait. After a QTL has been identified, it can be studied for the presence of one or more specific genes or other sequences

that influence the quantitative trait. The introduction to this chapter describes how this approach was used to identify a gene that affects oil production in corn. QTL mapping has been used to detect genes affecting a variety of characteristics in plant and animal species (Table 24.3).



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24.20 Mapping quantitative trait loci by linkage analysis can identify genes that help determine differences in quantitative traits. Genotypes at the *C* locus are

associated with the inheritance of differences in plant height, indicating that a QTL for height (the *T* locus) is closely linked to the *C* locus.

Description

The illustration shows P generation at the top. The P generation shows a tall plant (20 c m) with the following alleles: two long rod shaped chromosomes with big A superscript 1 big B superscript 1 each; two small rod shaped chromosomes with big C superscript 1 big T superscript positive each. A caption reads, Alleles at the T locus influence plant height. The A, B, and C loci are genetic markers. A small plant (10 c m) with the following alleles: two long rod shaped chromosomes with big A superscript 2 big B superscript 2 each; two small rod shaped chromosomes with big C superscript 2 big T superscript 0 each. A caption reads, Cross two homozygous strains that differ in alleles at many loci.

These plants are crossed and F subscript 1 generation is obtained below with a plant of intermediate height (15 c m). This plant has the following alleles: one long rod shaped chromosomes with big A superscript 1 big B superscript 1 and one long rod shaped chromosome with big A superscript 2 big B superscript 2; one small rod shaped chromosomes with big C superscript 1 big T superscript positive and one small rod shaped chromosomes with big C superscript 2 big T superscript 0. A caption reads, Intercross the F subscript 1 to allow genes to recombine.

F subscript 2 generation is obtained below with many genotypes. A caption reads, Many different combinations of alleles appear in the F subscript 2. The progenies obtained are as follows:

A tall plant has genotype: two long rod shaped chromosomes with big A superscript 1 big B superscript 1 each; two small rod shaped chromosomes with big C superscript 1 big T superscript positive each.

A small plant has genotype: two long rod shaped chromosomes with big A superscript 1 big B superscript 1 each; two small rod shaped chromosomes with big C superscript 2 big T superscript 0 each.

A plant with intermediate height has genotype: one long rod shaped chromosomes with big A superscript 1 big B superscript 1, one long rod shaped chromosome with big A superscript 2 big B superscript 2, one small rod shaped chromosomes with big C

superscript 1 big T superscript positive, and one small rod shaped chromosomes with big C superscript 2 big T superscript 0.

A tall plant has genotype: one long rod shaped chromosomes with big A superscript 2 big B superscript 1, one long rod shaped chromosomes with big A superscript 2 big B superscript 2, two small rod shaped chromosomes with big C superscript 1 big T superscript positive each.

A tall plant has genotype: one long rod shaped chromosomes with big A superscript 1 big B superscript 2, one long rod shaped chromosomes with big A superscript 2 big B superscript 2, two small rod shaped chromosomes with big C superscript 1 big T superscript positive each.

A small plant with genotype: one long rod shaped chromosomes with big A superscript 1 big B superscript 2, one long rod shaped chromosomes with big A superscript 2 big B superscript 2, two small rod shaped chromosomes with big C superscript 2 big T superscript 0 each.

A line graph shows height (c m) plotted against genotype. The horizontal axis represents genotype and has the following points: big A superscript 1 big A superscript 1, big A superscript 1 big A superscript 2, and big A superscript 2 big A superscript 2. The vertical axis represents height (c m) and ranges from 5 to 20, in increments of 5. A horizontal line is present for all three genotypes corresponding to 10 on the vertical axis. Three points are marked on the line. A caption reads, Measure offspring for the quantitative trait and genotype for numerous genetic markers.

A line graph shows height (c m) plotted against genotype. The horizontal axis represents genotype and has the following points: big B superscript 1 big B superscript 1, big B superscript 1 big B superscript 2, and big B superscript 2 big B superscript 2. The vertical axis represents height (c m) and ranges from 5 to 20, in increments of 5. A horizontal line is present for all three genotypes corresponding to 8 on the vertical axis. Two points are present on the line and one is little below.

A line graph shows height (c m) plotted against genotype. The horizontal axis represents genotype and has the following points: big C superscript 1 big C superscript 1, big C superscript 1 big C superscript 2, and big C superscript 2 big C superscript 2. The vertical axis represents height (c m) and ranges from 5 to 20, in increments of 5. A negative sloped line is present for all three genotypes. The points are present at: 15, 10, 5 of the

vertical axis for the three points on horizontal axis respectively. A caption reads, A correlation between genotype and the quantitative trait indicates that the genetic marker is linked to the Q T L, in this case locus T.

TABLE 24.3 Examples of quantitative characteristics for which QTLs have been detected

Organism	Quantitative Characteristic
Tomato	Soluble solids
	Fruit mass
	Fruit pH
	Growth
	Leaflet shape
	Height
Corn	Height
	Leaf length
	Tiller number
	Glume hardness
	Grain yield

	Number of ears
	Thermotolerance
Common bean	Number of root nodules
Mung bean	Seed weight
Cow pea	Seed weight
Wheat	Preharvest sprout
Pig	Growth
	Length of small intestine
	Average back fat
	Abdominal fat
Mouse	Epilepsy
Rat	Hypertension
Dog	Body size

Data from S. D. Tanksley, Mapping polygenes, *Annual Review of Genetics* 27:218, 1993. QTLs for body size in dogs from A. R. Boyko et al., A simple genetic architecture underlies morphological variation in dogs, *PLoS Biology* 8: e1000451, 2010.

GENOME-WIDE ASSOCIATION STUDIES

The traditional method of identifying QTLs is to carry out crosses between varieties that differ in a quantitative trait and then genotype numerous progeny for many markers. Although effective, this method is slow and labor-intensive.

An alternative technique for identifying genes that affect quantitative traits is to conduct genome-wide association studies, which were introduced in Chapter 7. Unlike traditional linkage analysis, which examines the association of a trait with genetic markers among the *progeny of a cross*, genome-wide association studies look for associations between traits and genetic markers in a *biological population*, a group of interbreeding individuals. The presence of an association between genetic markers and a trait indicates that the genetic markers are closely linked to one or more genes that affect variation in the trait. Genome-wide association studies have been facilitated by the identification of single-nucleotide polymorphisms (SNPs), which are positions in the genome where individual organisms vary in a single base pair (see Section 20.1). Many SNPs have been identified in organisms through genome sequencing. Individual organisms can often be quickly and inexpensively genotyped for numerous SNPs, which provide the genetic markers necessary to conduct genome-wide association studies.

Genome-wide association studies have been widely used to locate genes that affect quantitative traits in humans, including disease susceptibility, obesity, intelligence, and height. A number of quantitative traits in plants have also been studied, including kernel composition, size, color and taste, disease resistance, and starch quality. Genome-wide association studies in domesticated animals have identified chromosomal regions affecting body weight, body composition, reproductive traits, hormone levels, hair characteristics, and behaviors.

CONCEPTS

The availability of numerous genetic markers revealed by molecular methods makes it possible to map chromosomal regions containing genes that contribute to polygenic characteristics. Genome-wide association studies locate genes that affect quantitative traits by detecting associations between genetic markers and a trait within a population of individuals.

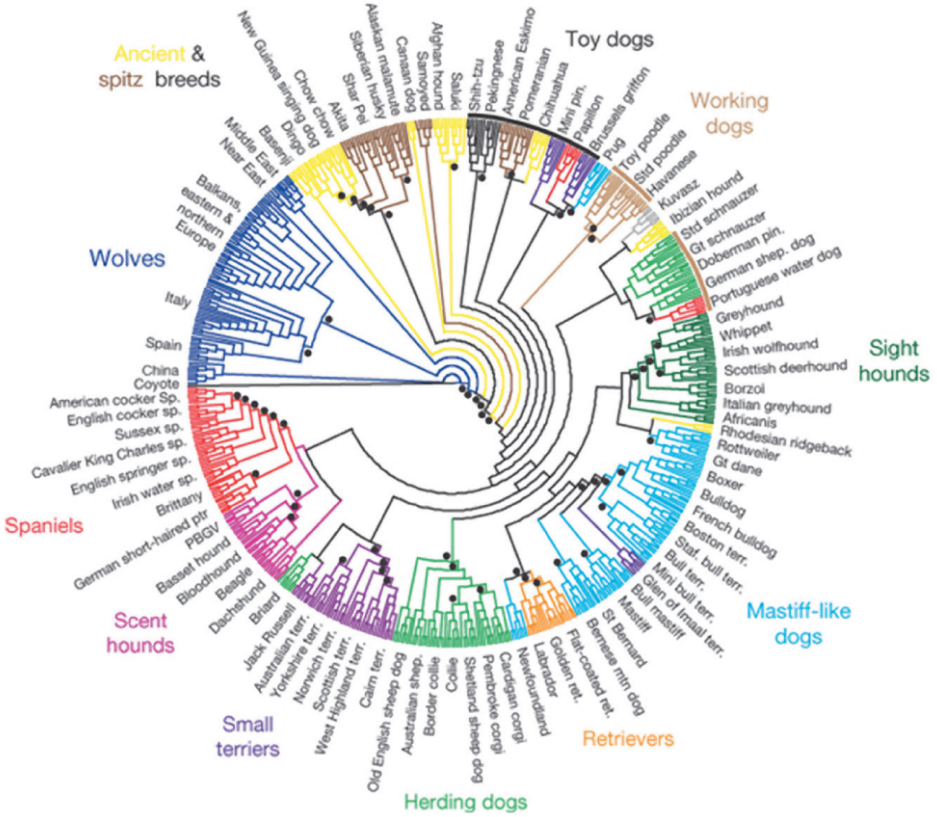
24.4 Genetically Variable Traits Change in Response to Selection

Evolution is genetic change that takes place among members of a population over time. Several different forces are potentially capable of bringing about evolution, and we will explore these forces and the process of evolution more fully in Chapter 25. Here, we consider how one of these forces—natural selection—can bring about genetic change in a quantitative characteristic.

Charles Darwin proposed the idea of natural selection in his book *On the Origin of Species* in 1859. **Natural selection** arises through the differential reproduction of individuals with different genotypes. Because of the genes they possess, some individuals produce more offspring than others. The more successful reproducers give rise to more offspring, which inherit the genes that confer a reproductive advantage. Thus, the frequencies of the genes that confer a reproductive advantage increase with the passage of time, and the population evolves. Natural selection is among the most important of the forces that bring about evolutionary change. Through natural selection, organisms become genetically suited to their environments; as environments change, groups of organisms change in ways that make them better able to survive and reproduce.

For thousands of years, humans have practiced a form of selection by promoting the reproduction of organisms with traits they

perceive as desirable. This form of selection, called **artificial selection**, has produced the domesticated plants and animals that make modern agriculture possible. The power of artificial selection—the first application of genetic principles by humans—is illustrated by the tremendous diversity of shapes, colors, and behaviors of modern domestic dogs (**Figure 24.21**).



B. M. von Holdt et al., Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication, *Nature* 464 (7290): 898–902. © 2010. Reprinted by permission from Macmillan Publishers Ltd. Permission conveyed through Copyright Clearance Center, Inc.

24.21 Artificial selection has produced the tremendous diversity of shape, size, color, and behavior seen today among breeds of domestic dogs. This diagram depicts the evolutionary relationships among wolves and different breeds of dogs as determined from analyses of DNA sequences.

Description
A circle shows the following breeds and sub breeds. Rising from common ancestors wolves are as follows: Spain, Italy, China, Middle East, near east, Balkans eastern and northern Europe.

Ancient and spitz breeds: Akita, Chow chow, Siberian husky, Canaan dog.

Toy dogs: Shih tzu, American Eskimo, Pomeranian, Chihuahua, Mini pin, Papillion, Pug.

Working dogs: Toy poodle, Std poodle, Havanese.

Sight hounds: Greyhound, whippet, Borzol, Italian greyhound, Africanis.

Mastiff like dogs: Boxer, bulldog, great Dane, French bulldog, Boston terr, Bull mastiff, mastiff, saint Bernard.

Retriever: Golden ret, Labrador, flat coated ret.

Herding dogs: Cardigan corgi, Pembroke Corgi, Australian shep, Old English sheep dog.

Small terriers: Yorkshire terr, Australian terr, Norwich terr, Scottish terr.

Scent hounds: Basset hound, blood hound, beagle, dachshund.

Spaniels: American cocker, English cocker, Sussex, English Springer, Brittany.

Revised Category (to be entered after TL's approval):

Page No. 754

Figure 24.22

Category 2

Predicting the Response to Selection

When a quantitative characteristic is subjected to natural or artificial selection, it frequently changes with the passage of time, provided that there is genetic variation for that characteristic in the population. Let's return to the dairy farmer who wants to increase milk production among the cows in the farmer's herd. Variation at several loci potentially affects milk production in cows; some alleles at these loci confer high milk production, whereas others confer low milk production. The dairy farmer breeds only those cows in the herd that have the highest milk production. If there is genetic variation for milk production in the herd (i.e., there are different alleles at the loci that control milk production), the mean milk production in the offspring of the selected cows should be higher than the mean milk production of the original herd. This increased production is due to the fact that the selected cows possess more alleles for high milk production than does the average cow, and these alleles are passed on to the offspring. Thus, the offspring of the selected cows possess a higher proportion of alleles for high milk production, and therefore produce more milk, than the average cow in the original herd.

The extent to which a characteristic subjected to selection changes in one generation is termed the **response to selection**. Suppose that the average cow in a dairy herd produces 80 liters of milk per week. A farmer selects for increased milk production by breeding the highest milk producers, and the female progeny of those selected cows produce 100 liters of milk per week on average. The response to selection is calculated by subtracting the mean phenotype of the

original population (80 liters) from the mean phenotype of the offspring (100 liters). In our example, the response to selection is 100 − 80 = 20 liters of milk per week.

FACTORS INFLUENCING RESPONSE TO SELECTION

The response to selection is determined primarily by two factors. First, it is affected by narrow-sense heritability, which largely determines the degree of resemblance between parents and offspring. When narrow-sense heritability is high, offspring will tend to resemble their parents; conversely, when narrow-sense heritability is low, there will be little resemblance between parents and offspring.

The second factor that determines the response to selection is how much selection there is. If the farmer is very stringent in the choice of parents and breeds only the highest milk producers in the herd (say, the top 2 cows), then all the offspring will receive genes for high milk production. If the farmer is less selective and breeds the top 20 milk producers in the herd, then the offspring will not carry as many genes for high milk production, and on average, they will not produce as much milk as the offspring of the top 2 producers. The response to selection depends on the phenotypic difference of the individuals that are selected as parents. This phenotypic difference is measured by the **selection differential**, defined as the difference between the mean phenotype of the selected parents and the mean phenotype of the original population. If the average milk

production of the original herd is 80 liters and the farmer breeds cows with an average milk production of 120 liters, then the selection differential is 120 − 80 = 40 liters.

CALCULATION OF RESPONSE TO SELECTION

The response to selection (R) depends on narrow-sense heritability (h^2) and the selection differential (S):

$$R = h^2 \times S \tag{24.21}$$

This equation can be used to predict the magnitude of change in a characteristic when a given selection differential is applied. G. A. Clayton and his colleagues estimated the response to selection that would take place in the abdominal bristle number of *Drosophila melanogaster*. By using several different methods, including parent–offspring regression, they first estimated the narrow-sense heritability of abdominal bristle number in a population of fruit flies to be 0.52. The mean number of bristles in the population was 35.3. They selected individual flies with a mean bristle number of 40.6 and intercrossed them to produce the next generation. The selection differential was 40.6 − 35.3 = 5.3, so they predicted that the response to selection would be

$$R = 0.52 \times 5.3 = 2.8$$

The response to selection of 2.8 is the expected increase in the phenotype of the offspring above the mean of the original population. They therefore expected the average number of abdominal bristles in the offspring of their selected flies to be 35.3 + 2.8 = 38.1. Indeed, they found an average bristle number of 37.9 in these flies.

ESTIMATING HERITABILITY FROM RESPONSE TO SELECTION

Rearranging Equation 24.21 provides another way to calculate narrow-sense heritability:

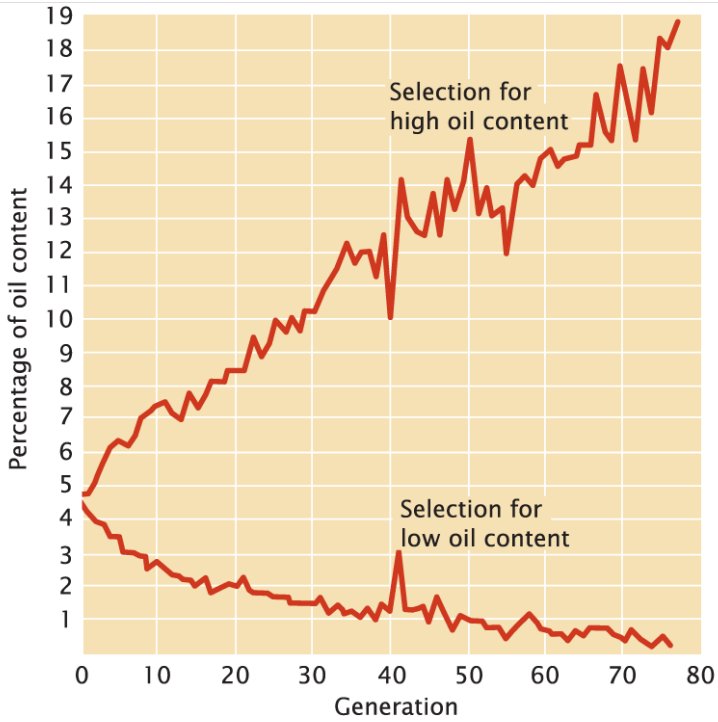
$$h^2 = \frac{R}{S} \tag{24.22}$$

In this way, h^2 can be calculated by conducting a response-to-selection experiment. First, the selection differential is obtained by subtracting the population mean from the mean of the selected parents. The selected parents are then interbred, and the mean phenotype of their offspring is measured. The difference between the mean of the offspring and that of the original population is the response to selection, which can be used with the selection differential to estimate heritability. Heritability determined by a response-to-selection experiment is usually termed **realized**

heritability. If certain assumptions are met, realized heritability is identical with narrow-sense heritability.

One of the longest-running selection experiments is a study of oil and protein content in corn kernels (**Figure 24.22**). This experiment began at the University of Illinois on 163 ears of corn with an oil content ranging from 4% to 6%. Corn plants with high oil content and corn plants with low oil content were selected and interbred. Response to selection for increased oil content (the upper line in Figure 24.22) reached about 20%, whereas response to selection for decreased oil content reached a lower limit near zero. Genetic analyses of the high- and low-oil-content strains revealed that at least 20 loci take part in determining oil content, one of which we explored in the introduction to this chapter. ❯ **TRY PROBLEM 42**

THINK-PAIR-SHARE Question 6



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

24.22 In a long-term response-to-selection experiment, selection for oil content in corn increased oil content in one line to about 20%, whereas it almost eliminated it in another line.

Description

The horizontal axis represents generation and ranges from 0 to 80, in increments of 10. The vertical axis represents percentage of oil content and ranges from 1 to 19, in increments of 1. A line labeled, selection for high oil content, begins from (0, 5), peaks below at (40, 10), and finally reaches (77, 19), after a large number of fluctuations. Another line labeled, selection for low oil content, begins at (0, 4 point 8), peaks at (40, 3), and finally reaches (77, 0), after a large number of fluctuations. All data are approximate.

CONCEPTS

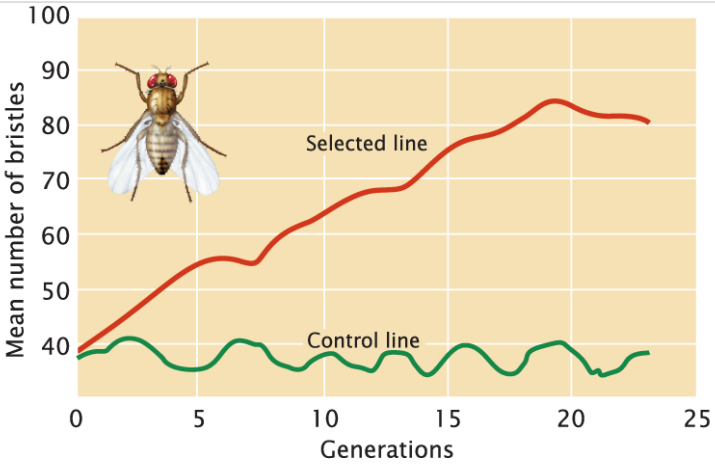
The response to selection is influenced by narrow-sense heritability and the selection differential.

✔ CONCEPT CHECK 7

The narrow-sense heritability for a trait is 0.4 and the selection differential is 0.5. What is the predicted response to selection?

Limits to the Response to Selection

When a characteristic has been selected for many generations, the response may eventually level off, and the characteristic may no longer respond to selection (Figure 24.23). A potential reason for this leveling off is that the genetic variation in the population is exhausted; at some point, all individuals in the population may have become homozygous for the alleles that encode the selected trait. When there is no more additive genetic variance, heritability equals zero, and no further response to selection can take place.



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24.23 The response of a population to selection often levels off at some point. In a response-to-selection experiment in which fruit flies were selected for increased bristle number in females, the number of bristles increased steadily for about 20 generations and then leveled off.

Description
The horizontal axis represents generation and ranges from 0 to 25, in increments of 5. The vertical axis represents mean number of bristles and ranges from 40 to 100, in increments of 10. A curve labeled, selected line, begins from (0, 40), peaks at (19, 84), and finally reaches (23, 80), after a few fluctuations. Another curve labeled, control line, begins at (0, 40) and finally reaches (23, 38), after a series of crests and troughs. All data are approximate.

Sometimes the response to selection levels off even while some genetic variation remains in the population. This leveling off takes place because natural selection opposes further change in the characteristic. The response to selection for small body size in mice, for example, eventually levels off because the smallest animals are sterile and cannot pass on their genes for small body size. In this case, artificial selection for small size is opposed by natural selection for fertility, and the population can no longer respond to the artificial selection.

THINK-PAIR-SHARE Question 7

Correlated Responses to Selection

Often when a specific trait is selected, other traits change at the same time. This type of associated response is due to the fact that the traits are encoded by the same genes.

Two or more characteristics are often correlated. For example, in many plants, plant size and number of seeds produced exhibit a positive correlation: larger plants, on average, produce more seeds than smaller plants. This type of correlation is called a **phenotypic correlation** because the association is between two phenotypes of the same individual. Phenotypic correlations may be due to environmental or genetic correlations. Environmental correlations refer to two or more characteristics that are influenced by the same environmental factor. Moisture availability, for example, may affect both the size of a plant and the number of seeds it produces. Plants growing in environments with lots of water are large and produce many seeds, whereas plants growing in environments with limited water are small and have few seeds.

Alternatively, a phenotypic correlation may result from a **genetic correlation**, which means that the genes affecting two characteristics are associated. The primary genetic cause of phenotypic correlations is pleiotropy, the effect of one gene on two or more characteristics (see Section 5.5). In humans, for example, many body structures respond to growth hormone, and there are genes that affect the amount of growth hormone secreted by the pituitary gland. People with certain genotypes produce high levels of growth hormone, which increases both height and hand size. Those

with other genotypes produce lower levels of growth hormone, which leads to both short stature and small hands. Height and hand size are therefore phenotypically correlated in humans, and this correlation is due to a genetic correlation—the fact that both characteristics are affected by the same genes that control the amount of growth hormone. Genetically speaking, height and hand size are the same characteristic because they are the phenotypic manifestation of a single set of genes. When two characteristics are influenced by the same genes, they are genetically correlated.

Genetic correlations are quite common (Table 24.4) and may be positive or negative. A positive genetic correlation between two characteristics means that genes that cause an increase in one characteristic also produce an increase in the other characteristic. Thorax length and wing length in *Drosophila* are positively correlated because the genes that increase thorax length also increase wing length. A negative genetic correlation means that genes that cause an increase in one characteristic produce a decrease in the other characteristic. Milk yield and percentage of butterfat are negatively correlated in cattle: genes that cause higher milk production result in milk with a lower percentage of butterfat.

TABLE 24.4 Genetic correlations in various organisms

Organism	Characteristics	Genetic Correlation
Cattle	Milk yield and percentage of	−0.38

	butterfat	
Pig	Weight gain and back-fat thickness	0.13
	Weight gain and efficiency	0.69
Chicken	Body weight and egg weight	0.42
	Body weight and egg production	−0.17
	Egg weight and egg production	−0.31
Mouse	Body weight and tail length	0.29
Fruit fly	Abdominal bristle number and sternopleural bristle number	0.41

Data from D. S. Falconer and T. F. C. Mackay, *Introduction to Quantitative Genetics* (New York: Pearson, 1996), p. 314.

Genetic correlations are important in animal and plant breeding because they produce a correlated response to selection, which means that when one characteristic is selected, genetically correlated characteristics also change. Correlated responses to selection are due to the fact that both characteristics are influenced

by the same genes; selection for one characteristic causes a change in the genes affecting that characteristic, and because those genes also affect the second characteristic, they cause it to change at the same time. Correlated responses may well be undesirable and may limit our ability to alter a characteristic by selection. From 1944 to 1964, for example, domesticated turkeys were subjected to intense selection for growth rate and body size. At the same time, fertility, egg production, and egg hatchability all declined. These correlated responses were due to negative genetic correlations between body size and fertility. Eventually, these genetic correlations limited the extent to which the growth rate of turkeys could respond to selection. Genetic correlations may also limit the ability of natural populations to respond to selection in the wild and adapt to their environments. › **TRY** PROBLEM 47

CONCEPTS

Genetic correlations result from pleiotropy. When two characteristics are genetically correlated, selection for one characteristic will produce a correlated response in the other characteristic.

✔ CONCEPT CHECK 8

In a herd of dairy cattle, milk yield and the percentage of butterfat exhibit a genetic correlation of −0.38. If greater milk yield is selected in this herd, what will be the effect on the percentage of butterfat?

Chapter 24 Review

CHAPTER SUMMARY

Section 24.1 Learning Objective: Be able to summarize the differences between continuous and discontinuous characteristics.

- Quantitative genetics focuses on the inheritance of complex characteristics whose phenotypes vary continuously. For many quantitative characteristics, the relation between genotype and phenotype is complex because many genes, as well as environmental factors, influence a characteristic.
- The individual genes that influence a polygenic characteristic follow the same Mendelian principles that govern discontinuous characteristics, but because many genes participate, the expected ratios of phenotypes are obscured.

Section 24.2 Learning Objective: Be able to summarize statistical methods used for analyzing quantitative characteristics.

- A frequency distribution, in which phenotypes are represented on one axis and the numbers or proportions of individuals possessing each phenotype are represented on the other axis, is a convenient means of summarizing the phenotypes found in a group of individuals.

- A population is the group of interest, and a sample is a subset of the population used to describe it.
- The mean and variance provide key information about a distribution: the mean gives the central location of the distribution, and the variance provides information about how the phenotype varies within a group.
- The correlation coefficient measures the direction and strength of association between two variables. Regression can be used to predict the value of one variable on the basis of the value of a correlated variable.

Section 24.3 Learning Objective: Be able to explain the concept of heritability, how it is calculated, and its limitations.

- Phenotypic variance in a characteristic can be divided into components that are due to additive genetic variance, dominance genetic variance, gene interaction variance, environmental variance, and genetic–environmental interaction variance.
- Broad-sense heritability is the proportion of phenotypic variance that is due to genetic variance. Narrow-sense heritability is the proportion of phenotypic variance that is due to additive genetic variance.
- Heritability provides information only about the degree to which variation in a characteristic results from genetic differences. Heritability is based on the variation present within a group of individuals; an individual does not have heritability. The heritability of a characteristic varies among populations

and among environments. Even if the heritability for a characteristic is high, the characteristic may still be altered by changes in the environment. Heritabilities provide no information about the nature of differences among populations in a characteristic.

- Quantitative trait loci (QTLs) are chromosomal regions containing genes that influence polygenic characteristics. QTLs can be mapped by examining the association between the inheritance of a quantitative characteristic and the inheritance of genetic markers. Genes influencing quantitative traits can also be located with the use of genome-wide association studies.

Section 24.4 Learning Objective: Be able to explain response to selection and how it is calculated.

- The amount of change in a quantitative characteristic in a single generation when subjected to selection (the response to selection) is directly related to the selection differential and narrow-sense heritability.
- A genetic correlation may be present when the same gene affects two or more characteristics (pleiotropy). Genetic correlations produce correlated responses to selection.

WORKED PROBLEMS

Problem 1

Seed weight in a particular plant species is determined by pairs of alleles at two loci (a^+a^- and b^+b^-) that are additive and equal in their effects. Plants with genotype $a^-a^-\ b^-b^-$ have seeds that average 1 g in weight, whereas plants with genotype $a^+a^+\ b^+b^+$ have seeds that average 3.4 g in weight. A plant with genotype $a^-a^-\ b^-b^-$ is crossed with a plant of genotype $a^+a^+\ b^+b^+$.

a. What is the predicted weight of seeds from the F_1 progeny of this cross?
b. If the F_1 plants are intercrossed, what are the expected seed weights and proportions of the F_2 plants?

» Solution Strategy

What information is required in your answer to the problem?

- The predicted weight of seeds from the F_1 progeny
- The expected seed weights and their proportions among the F_2 progeny

What information is provided to solve the problem?

- Seed weight is determined by pairs of alleles at two loci (a^+a^- and b^+b^-).
- The alleles are additive and equal in their effects.
- Plants with genotype $a^-a^-\ b^-b^-$ have seeds that average 1 g in weight.

- Plants with genotype $a^+a^+\ b^+b^+$ have seeds that average 3.4 g in weight.
- A plant with genotype $a^-a^-\ b^-b^-$ is crossed with a plant of genotype $a^+a^+\ b^+b^+$.

For help with this problem, review:

Polygenic Inheritance in Section 24.1.

» Solution Steps

The difference in average seed weight between the two parental genotypes is 3.4 g − 1 g = 2.4 g. These two genotypes differ in four genes, so each gene difference contributes an additional 2.4 g/4 = 0.6 g of weight to the 1 g seed weight of a plant ($a^-a^-\ b^-b^-$) that has none of the contributing genes.

> **Hint:** Because the alleles are equal and additive, each allele contributes the same amount to seed weight.

The cross between the two homozygous genotypes produces the F_1 and F_2 progeny shown below.

a. The F_1 are heterozygous at both loci ($a^+a^-\ b^+b^-$) and possess two genes that contribute an additional 0.6 g each to the 1 g seed weight of a plant that has no contributing genes. Therefore, the seeds of the F_1 should average 1 g + 2(0.6 g) = 2.2 g.

b. The F_2 have the following phenotypes and proportions: $^1/_{16}$1 g, $^4/_{16}$1.6 g, $^6/_{16}$2.2 g, $^4/_{16}$2.8 g, and $^1/_{16}$3.4 g.

P $a^-a^-\ b^-b^-$ (1 g) × $a^+a^+\ b^+b^+$ (3.4 g)

↓

F_1 $a^+a^-\ b^+b^-$ (2.2 g)

↓

	Genotype	Probability		Number of contributing genes	Average seed weight
	$a^-a^-\ b^-b^-$	$1/4 \times 1/4 = 1/16$		0	$1\text{ g} + (0 \times 0.6\text{ g}) = 1\text{ g}$
	$a^+a^-\ b^-b^-$	$1/2 \times 1/4 = 1/8$	$2/8 = 4/16$	1	$1\text{ g} + (1 \times 0.6\text{ g}) = 1.6\text{ g}$
	$a^-a^-\ b^+b^-$	$1/4 \times 1/2 = 1/8$			
F_2	$a^+a^+\ b^-b^-$	$1/4 \times 1/4 = 1/16$			
	$a^-a^-\ b^+b^+$	$1/4 \times 1/4 = 1/16$	$2/16 + 1/4 = 6/16$	2	$1\text{ g} + (2 \times 0.6\text{ g}) = 2.2\text{ g}$
	$a^+a^-\ b^+b^-$	$1/2 \times 1/2 = 1/4$			
	$a^+a^+\ b^+b^-$	$1/4 \times 1/2 = 1/8$	$2/8 = 4/16$	3	$1\text{ g} + (3 \times 0.6\text{ g}) = 2.8\text{ g}$
	$a^+a^-\ b^+b^+$	$1/4 \times 1/2 = 1/8$			
	$a^+a^+\ b^+b^+$	$1/4 \times 1/4 = 1/16$		4	$1\text{ g} + (4 \times 0.6\text{ g}) = 3.4\text{ g}$

Description

The P generation at the top shows small A superscript negative small A superscript negative small B superscript negative small B superscript negative (1 g) crossed with small A superscript positive small A superscript positive small B superscript positive small B superscript positive (3 point 4 g). The F subscript 1 generation obtained below shows small A superscript positive small A superscript negative small B superscript positive small B superscript negative (2 point 2 g). A table below shows F subscript 2 generation. The table has 5 rows and 4 columns. The column headers are as follows: Genotype, Probability, Number of contributing genes, and Average seed weight. The information from the table is as follows:

Row 1: small A superscript negative small A superscript negative small B superscript negative small B superscript negative; one fourth multiplied by one fourth equals one-sixteenth; 0; 1 g plus open parenthesis 0 multiplied by 0 point 6 g close parenthesis equals 1 g.

Row 2: small A superscript positive small A superscript negative small B superscript negative small B superscript negative and small A superscript negative small A superscript

negative small B superscript positive small B superscript negative; one half multiplied by one fourth equals one eighth and one fourth multiplied by one half equals one eighth, the collective probability is two eighth equals four sixteenth; 1; 1 g plus open parenthesis 1 multiplied by 0 point 6 g close parenthesis equals 1 point 6 g.

Row 3: small A superscript positive small A superscript positive small B superscript negative small B superscript negative, small A superscript positive small A superscript negative small B superscript positive small B superscript positive, and small A superscript positive small A superscript negative small B superscript positive small B superscript negative; one fourth multiplied by one fourth equals one sixteenth, one fourth multiplied by one fourth equals one sixteenth, one half multiplied by one half equals one fourth, the collective probability is two sixteenth plus one fourth equals six sixteenth; 2; 1 g plus open parenthesis 2 multiplied by 0 point 6 g close parenthesis equals 2 point 2 g.

Row 4: small A superscript positive small A superscript positive small B superscript positive small B superscript negative and small A superscript positive small A superscript negative small B superscript positive small B superscript positive; one fourth multiplied by one half equals one eighth, one fourth multiplied by one half equals one eighth, the collective probability is two eighth equals four sixteenth; 3; 1 g plus open parenthesis 3 multiplied by 0 point 6 g close parenthesis equals 2 point 8 g.

Row 5: small A superscript positive small A superscript positive small B superscript positive small B superscript positive; one fourth multiplied by one fourth equals one sixteenth; 4; 1 g plus open parenthesis 4 multiplied by 0 point 6 g close parenthesis equals 3 point 4 g.

Recall: The probability of each two-locus genotype can be determined by multiplying the probability of the single-locus genotypes.

Problem 2

A farmer is raising rabbits. The average body weight in her population of rabbits is 3 kg. The farmer selects the 10 largest

rabbits in the population, whose average body weight is 4 kg, and interbreeds them. If the narrow-sense heritability of body weight in the rabbit population is 0.7, what is the expected body weight among the offspring of the selected rabbits?

» Solution Strategy

What information is required in your answer to the problem?

The expected body weight of the offspring of the selected rabbits.

What information is provided to solve the problem?

- The average body weight in the population is 3 kg.
- The average body weight of selected rabbits is 4 kg.
- The narrow-sense heritability of body weight is 0.7.

For help with this problem, review:

Predicting the Response to Selection in Section 24.4.

» Solution Steps

The farmer has carried out a response-to-selection experiment. The selection differential equals the difference in average weight between the selected rabbits and the entire population: 4 kg − 3 kg = 1 kg. The narrow-sense heritability is given as 0.7, so the expected

response to selection is $R = h^2 \times S = 0.7 \times 1$ kg = 0.7 kg. This value is the increase in weight that is expected in the offspring of the selected parents, so the average weight of the offspring is expected to be 3 kg + 0.7 kg = 3.7 kg.

Recall: The response to selection equals the selection differential multiplied by narrow-sense heritability.

IMPORTANT TERMS

quantitative genetics
meristic characteristic
threshold characteristic
frequency distribution
normal distribution
population
sample
mean
variance
standard deviation
correlation
correlation coefficient
regression
regression coefficient
heritability
phenotypic variance
genetic variance

environmental variance
genetic–environmental interaction variance
additive genetic variance
dominance genetic variance
gene interaction variance
broad-sense heritability
narrow-sense heritability
quantitative trait locus (QTL)
natural selection
artificial selection
response to selection
selection differential
realized heritability
phenotypic correlation
genetic correlation

ANSWERS TO CONCEPT CHECKS

1. Cross two individuals that are each homozygous for different genes affecting the traits and then intercross the resulting F_1 progeny to produce the F_2. Determine what proportion of the F_2 progeny resemble one of the original homozygotes in the P generation. This proportion should be $(1/4)^n$, where n equals the number of loci with segregating pairs of alleles that affect the characteristic.
2. d
3. d

4. b
5. a
6. It indicates that about 40% of the differences in blood pressure among African Americans in Detroit are due to additive genetic differences. It neither provides information about the heritability of blood pressure in other groups of people nor indicates anything about the nature of differences in blood pressure between African Americans in Detroit and people in other groups.
7. 0.2
8. The percentage of butterfat will decrease.

Chapter 24 Assessment

COMPREHENSION QUESTIONS

Section 24.1

1. How does a quantitative characteristic differ from a discontinuous characteristic?

2. Briefly explain why the relation between genotype and phenotype is frequently complex for quantitative characteristics.

3. Why do polygenic characteristics have many phenotypes?

Section 24.2

4. Explain the relation between a population and a sample. What characteristics should a sample have to be representative of the population?

5. What information do the mean and variance provide about a distribution?

6. How is the standard deviation related to the variance?

7. What information does the correlation coefficient provide about the association between two variables?

8. What is regression? How is it used?

Section 24.3

9. List all the components that contribute to the phenotypic variance, and define each component.

10. How do broad-sense and narrow-sense heritabilities differ?

11. Briefly outline some of the ways in which heritability can be calculated.

12. Briefly describe common misunderstandings or misapplications of the concept of heritability.

13. Briefly explain how genes affecting a polygenic characteristic are located with the use of QTL mapping.

Section 24.4

14. How is the response to selection related to narrow-sense heritability and the selection differential? What information does the response to selection provide?

15. Why does the response to selection often level off after many generations of selection?

APPLICATION QUESTIONS AND PROBLEMS

Section 24.1

16. *For each of the following characteristics, indicate whether it would be considered a discontinuous characteristic or a quantitative characteristic. Briefly justify your answer.
    a. Kernel color in a strain of wheat, in which two codominant alleles segregating at a single locus determine the color. Thus, there are three phenotypes present in this strain: white, light red, and medium red.
    b. Body weight in a family of Labrador retrievers. An autosomal recessive allele that causes dwarfism is present in this family. Two phenotypes are recognized: dwarf (less than 13 kg) and normal (greater than 23 kg).
    c. Presence or absence of leprosy. Susceptibility to leprosy is determined by multiple genes and numerous environmental factors.
    d. Number of toes in guinea pigs, which is influenced by genes at many loci.
    e. Number of fingers in humans. Extra (more than five) fingers are caused by the presence of an autosomal dominant allele.

17. CONNECT What do the principle of segregation and the principle of independent assortment (see Chapter 3) indicate about

heredity? Do these principles apply to quantitative characteristics? Why or why not?

18. *Assume that plant weight is determined by a pair of alleles at each of two independently assorting loci (*A* and *a*, *B* and *b*) that are additive in their effects. Further assume that each allele represented by an uppercase letter contributes 4 g to weight and that each allele represented by a lowercase letter contributes 1 g to weight.
    a. If a plant with genotype *AA BB* is crossed with a plant with genotype *aa bb*, what weights are expected in the F_1 progeny?
    b. What is the distribution of weights expected in the F_2 progeny?

19. Assume that three loci, each with two alleles (*A* and *a*, *B* and *b*, and *C* and *c*), determine the difference in height between two homozygous strains of a plant. These genes are additive and equal in their effects on plant height. One strain (*aa bb cc*) is 10 cm in height. The other strain (*AA BB CC*) is 22 cm in height. The two strains are crossed, and the resulting F_1 are interbred to produce F_2 progeny. Give the phenotypes and the expected proportions of the F_2 progeny.

20. *A farmer has two homozygous varieties of tomatoes. One variety, called Little Pete, has fruits that average only 2 cm in diameter. The other variety, Big Boy, has fruits that average a whopping 14 cm in diameter. The farmer crosses Little Pete and Big Boy; he then intercrosses the F_1 to produce F_2 progeny.

He grows 2000 F_2 tomato plants and doesn't find any F_2 offspring that produce fruits as small as Little Pete or as large as Big Boy. If we assume that the difference between these varieties in fruit size is produced by genes with equal and additive effects, what can we conclude about the minimum number of loci with pairs of alleles determining the difference in fruit size between the two varieties?

21. Seed size in a plant is a polygenic characteristic. A grower crosses two pure-breeding varieties of the plant and measures seed size in the F_1 progeny. She then backcrosses the F_1 plants to one of the parental varieties and measures seed size in the backcross progeny. The grower finds that seed size in the backcross progeny has a higher variance than does seed size in the F_1 progeny. Explain why the backcross progeny are more variable.

22.

One of the traits Mendel studied was height in pea plants. In Mendel's peas, height was a discontinuous characteristic with two phenotypes, tall and short. The phenotypes were determined by alleles at a single locus, with the allele for tall dominant to the allele for short (see Chapter 3). Imagine that height in Mendel's peas had been a quantitative trait with a threshold characteristic, so that there were still only two phenotypes, tall and short, but these phenotypes were encoded by alleles at multiple loci. Would this have affected the results of Mendel's crosses?

Do you think it would have influenced the conclusions Mendel reached?

Section 24.2

23. The following data are the numbers of digits per foot in 25 guinea pigs. Construct a frequency distribution for these data.

$$4, 4, 4, 5, 3, 4, 3, 4, 4, 5, 4, 4, 3, 2, 4, 4, 5, 6, 4, 4, 3, 4, 4, 4, 5$$

24. *Ten male Harvard students were weighed in 1916. Their weights are given here in kilograms. Calculate the mean, variance, and standard deviation for these weights.

$$51, 69, 69, 57, 61, 57, 75, 105, 69, 63$$

25. Among a population of tadpoles, the correlation coefficient for size at metamorphosis and time required for metamorphosis is −0.74. On the basis of this correlation, what conclusions can you draw about the relative sizes of tadpoles that metamorphose quickly and those that metamorphose more slowly?

26. *Body weight and length were measured on six mosquito fish; these measurements are given in the following table. Calculate the correlation coefficient for weight and length in these fish.

A. Hartl/AgeFotostock.

Wet weight (g)	Length (mm)
115	18
130	19
210	22
110	17
140	20
185	21

27. *The heights of mothers and daughters are given in the following table:

Height of mother (in)	Height of daughter (in)
64	66
65	66

66	68
64	65
63	65
63	62
59	62
62	64
61	63
60	62

a. Calculate the correlation coefficient for the heights of the mothers and daughters.
b. Using regression, predict the expected height of a daughter whose mother is 67 inches tall.

Section 24.3

28. *Phenotypic variation in the tail length of mice has the following components:

Additive genetic variance (V_A)	= 0.5

Dominance genetic variance (V_D)	= 0.3
Gene interaction variance (V_I)	= 0.1
Environmental variance (V_E)	= 0.4
Genetic–environmental interaction variance (V_{GE})	= 0.0

a. What is the narrow-sense heritability of tail length?
b. What is the broad-sense heritability of tail length?

29. The narrow-sense heritability of ear length in Reno rabbits is 0.4. The phenotypic variance (V_P) is 0.8, and the environmental variance (V_E) is 0.2. What is the additive genetic variance (V_A) for ear length in these rabbits?

30. Assume that human ear length is influenced by multiple genetic and environmental factors. Suppose you measure ear length in three groups of people, in which group A consists of five unrelated people, group B consists of five siblings, and group C consists of five first cousins.
    a. With the assumption that the environments of all three groups are similar, which group should have the highest phenotypic variance? Explain why.
    b. Is it realistic to assume that the environmental variance for each group is similar? Explain your answer.

31. A characteristic has a narrow-sense heritability of 0.6.

a. If the dominance variance (V_D) increases and all other variance components remain the same, what will happen to narrow-sense heritability? Will it increase, decrease, or remain the same? Explain.
b. What will happen to broad-sense heritability? Explain.
c. If the environmental variance (V_E) increases and all other variance components remain the same, what will happen to narrow-sense heritability? Explain.
d. What will happen to broad-sense heritability? Explain.

32. Flower color in the varieties of pea plants studied by Mendel is controlled by alleles at a single locus. A group of peas homozygous for purple flowers is grown. Careful study of the plants reveals that all their flowers are purple, but there is some variation in the intensity of the purple color. What would the estimated heritability be for this variation in flower color? Explain your answer.

33. *A graduate student is studying a population of bluebonnets along a roadside. The plants in this population are genetically variable. She counts the seeds produced by each of 100 plants and measures the mean and variance of seed number. The variance is 20. Selecting one plant, the student takes cuttings from it and cultivates them in a greenhouse, eventually producing many genetically identical clones of the same plant. She then transplants these clones into the roadside population, allows them to grow for one year, and then counts the seeds produced by each of the cloned plants. The student finds that the variance in seed number among these cloned plants is 5.

From the phenotypic variances of the genetically variable and the genetically identical plants, she calculates the broad-sense heritability.

Purestock/Getty Images.

a. What is the broad-sense heritability of seed number for the roadside population of bluebonnets?
b. What might cause this estimate of heritability to be inaccurate?

34. Many researchers have estimated the heritability of human traits by comparing the correlation coefficients of monozygotic and dizygotic twins (see pp. 747–748). One of the assumptions made in using this method is that monozygotic twin pairs

experience environments that are no more similar to each other than those experienced by dizygotic twin pairs. How might this assumption be violated? Give some specific examples of how the environments of two monozygotic twins might be more similar than the environments of two dizygotic twins.

35. What conclusion can you draw from **Figure 24.18** about the proportion of phenotypic variation in shell breadth that is due to genetic differences? Explain your reasoning.

36. *A genetics researcher determines that the broad-sense heritability of height among Southwestern University undergraduate students is 0.90. Which of the following conclusions would be reasonable? Explain your answer.
    a. Sally is a Southwestern University undergraduate student, so 10% of her height is determined by nongenetic factors.
    b. Ninety percent of variation in height among all undergraduate students in the United States is due to genetic differences.
    c. Ninety percent of the height of Southwestern University undergraduate students is determined by genes.
    d. Ten percent of the variation in height among Southwestern University undergraduate students is determined by variation in nongenetic factors.
    e. Because the heritability of height among Southwestern University undergraduate students is so high, any change in the students' environment will have minimal effect on their height.

37. *The length of the middle joint of the right index finger was measured in 10 sets of parents and their adult offspring. The mean parental lengths and the mean offspring lengths for each family are listed in the accompanying table. Calculate the regression coefficient for regression of mean offspring length against mean parental length, and estimate the narrow-sense heritability for this characteristic.

Mean parental length (mm)	Mean offspring length (mm)
30	31
35	36
28	31
33	35
26	27
32	30
31	34
29	28
40	38

38. Assume that in **Figure 24.14**, *x* equals the mean phenotype of the parents and *y* equals the mean phenotype of the offspring. Which line represents the highest heritability? Explain your answer.

39. *Drosophila buzzatii* is a fruit fly that feeds on the rotting fruits of cacti in Australia. Timothy Prout and Stuart Barker calculated the heritabilities of body size, as measured by thorax length, for a natural population of *D. buzzatii* raised in the wild and for a population of *D. buzzatii* collected in the wild but raised in the laboratory (T. Prout and J. S. F. Barker. 1989. *Genetics* 123:803–813). They found the following heritabilities:

Population	Heritability of body size (± standard error)
Wild population	0.0595 ± 0.0123
Laboratory-reared population	0.3770 ± 0.0203

Why do you think that the heritability measured in the laboratory-reared population is higher than that measured in the natural population raised in the wild?

40. Mr. Jones is a pig farmer. For many years, he has fed his pigs the food left over from the local university cafeteria, which is known to be low in protein, deficient in vitamins, and downright untasty. However, the food is free, and his pigs don't complain. One day a salesman from a feed company visits Mr. Jones. The salesman claims that his company sells a new high-protein, vitamin-enriched feed that enhances weight gain in pigs. Although the feed is expensive, the salesman claims that the increased weight gain of the pigs will more than pay for the cost of the feed, increasing Mr. Jones's profit. Mr. Jones responds that he took a genetics class at the university and that he has conducted some genetic experiments on his pigs; specifically, he has calculated the narrow-sense heritability of weight gain for his pigs and found it to be 0.98. Mr. Jones says that this heritability value indicates that 98% of the variance in weight gain among his pigs is determined by genetic differences, and therefore the new pig feed would have little effect on the growth of his pigs. He concludes that the feed would be a waste of his money. The salesman doesn't dispute Mr. Jones's heritability estimate, but he still claims that the new feed could significantly increase weight gain in Mr. Jones's pigs. Who is correct, and why?

41.

How is QTL mapping similar to or different from genome-wide association studies? (For more information about genome-wide association studies, see Chapter 20.)

Section 24.4

42. *Joe is breeding cockroaches in his dorm room. He finds that the average wing length in his population of cockroaches is 4 cm. He chooses the six cockroaches that have the largest wings; the average wing length among these selected cockroaches is 10 cm. Joe interbreeds these selected cockroaches. From earlier studies, he knows that the narrow-sense heritability for wing length in his population of cockroaches is 0.6.
    a. Calculate the selection differential and expected response to selection for wing length in these cockroaches.
    b. What should be the average wing length of the progeny of the selected cockroaches?

43. Three characteristics in beef cattle—body weight, fat content, and tenderness—are measured, and the following variance components are estimated:

	Body weight	**Fat content**	**Tenderness**
V_A	22	45	12
V_D	10	25	5
V_I	3	8	2
V_E	42	64	8

V_{GE}	0	0	1

In this population, which characteristic would respond best to selection? Explain your reasoning.

44. A rancher determines that the average amount of wool produced by a sheep in her flock is 22 kg per year. In an attempt to increase the wool production of her flock, the rancher picks the five male and five female sheep that produce the most wool; the average amount of wool produced per sheep by those selected sheep is 30 kg. She interbreeds these selected sheep and finds that the average wool production among their progeny is 28 kg. What is the narrow-sense heritability for wool production among the sheep in the rancher's flock?

45. A strawberry farmer determines that the average weight of individual strawberries produced by plants in his garden is 2 g. He selects the 10 plants that produce the largest strawberries; the average weight of strawberries produced by these selected plants is 6 g. He interbreeds these selected plants. The progeny of these selected plants produce strawberries that weigh an average of 5 g. If the farmer were to select plants that produce strawberries with an average weight of 4 g, what would be the predicted weight of strawberries produced by the progeny of those selected plants?

46. Has the response to selection leveled off in the strain of corn selected for high oil content shown in **Figure 24.22**? What does

this observation suggest about genetic variation in the strain selected for high oil content?

47. *The narrow-sense heritability of wing length in a population of *Drosophila melanogaster* is 0.8. The narrow-sense heritability of head width in the same population is 0.9. The genetic correlation between wing length and head width is −0.86. If a geneticist selects for increased wing length in these flies, what will happen to head width?

48. Pigs have been domesticated from wild boars. Would you expect to find higher heritability for weight among domesticated pigs or wild boars? Explain your answer.

49. CONNECT The fitness of a genotype is its reproductive success relative to other genotypes in a population (see Chapter 25). If the fitnesses of all genotypes in a population were the same, what would be the response to selection?

CHALLENGE QUESTIONS

Section 24.1

50. Bipolar disorder is a psychiatric illness with a strong hereditary basis, but the exact mode of its inheritance is not known. Research has shown that siblings of patients with bipolar

disorder are more likely to develop the disorder than are siblings of unaffected people. Findings from one study demonstrated that the ratio of bipolar brothers to bipolar sisters is higher when the patient is male than when the patient is female. In other words, relatively more brothers of patients with bipolar disorder also have the disease when the patient is male than when the patient is female. What does this observation suggest about the inheritance of bipolar disorder?

Section 24.3

51. We have explored some of the difficulties in separating the genetic and environmental components of human behavioral characteristics. Considering these difficulties and what you know about calculating heritability, propose an experimental design for accurately measuring the heritability of musical ability.

Section 24.4

52.

Eugene Eisen selected for increased 12-day litter weight (total weight of a litter of offspring 12 days after birth) in a population of mice (E. J. Eisen. 1972. *Genetics* 72:129–142). The 12-day litter weight of the population steadily increased but then leveled off after about 17 generations. At generation 17, Eisen took one family of mice from the selected population and reversed the selection procedure: in this group, he selected for *decreased* 12-day litter weight. This group immediately responded to the reversed selection: the 12-day litter weight

dropped 4.8 g within 1 generation and dropped 7.3 g after 5 generations. On the basis of the results of the reverse selection, what is the most likely explanation for the leveling off of 12-day litter weight in the original population?

J & C Sohns/Age Fotostock America, Inc.

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 24.1

1. To locate genes associated with quantitative traits, geneticists often use QTL mapping (see the introduction to this chapter). To carry out QTL mapping, a first step is crossing two strains that differ in a quantitative trait, such as a strain of corn with high oil content and a strain with low oil content. The F_1 progeny of this cross are then interbred or backcrossed to

produce an F_2 generation. Researchers then look for statistical associations between genetic markers and the value of the quantitative trait (e.g., oil content) in the F_2 generation. Why do the geneticists look for statistical associations in the F_2 plants? Why not use the F_1 progeny?

Section 24.2

2. What is the value of a random sample? Will random sampling always ensure that a sample is representative of a population? Explain your answer.

3. A researcher studying alcohol consumption in North American cities finds a significant correlation coefficient of 0.85 between the number of preachers in a city and total alcohol consumption for the city. Is it reasonable for the researcher to conclude that the preachers are consuming most of the alcohol? Why or why not? If not, how would you account for the significant correlation?

Section 24.3

4. A student who has just learned about quantitative genetics says, “Heritability estimates are worthless! They don’t tell you anything about the genes that affect a characteristic. They don’t provide any information about the types of offspring to expect from a cross. Heritability estimates measured in one population can’t be used for other populations, so they don’t even give you any general information about how much of a

characteristic is genetically determined. Heritabilities don't do anything but make undergraduate students sweat during tests." How would you respond to this statement? Is the student correct? What good are heritabilities, and why do geneticists bother to calculate them?

5. Do you think genetics influences a person's knowledge of the game of football? Explain your answer.

Section 24.4

6. We have learned that the response to selection is equal to the selection differential times the narrow-sense heritability, and that the narrow-sense heritability includes only the additive genetic variance. Why aren't the dominance genetic variance and the gene interaction variance included? Why don't they contribute to the genetic variation that is acted on by selection?

7. A geneticist selects for increased body weight in a population of fruit flies that she is raising in her laboratory. She measures body weight in her population and selects the five heaviest males and the five heaviest females and uses them as the parents for the next generation. From the progeny produced by these parents, she selects the five heaviest males and the five heaviest females and mates them. She repeats this procedure each generation. The average body weight of flies in the original population was 1.1 mg. The flies respond to selection, and their body weight steadily increases. After 20 generations of selection, the average body weight is 2.3 mg. However, after

about 20 generations, the response to selection in subsequent generations levels off, and the average body weight of the flies no longer increases. At this point, the geneticist takes a long vacation; while she is gone, the fruit flies in her population interbreed randomly. When she returns from vacation, she finds that the average body weight of the flies in the population has decreased to 2.0 mg.

Provide an explanation for why the response to selection leveled off after 20 generations. Why did the average size of the fruit flies decrease when selection was no longer applied during the geneticist's vacation?

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Draw a concept map that outlines how QTL analysis and fine-scale mapping of QTLs led to the discovery of a gene that influences oil content in corn. Include at least the following terms: QTLs, oil content of corn, polygenic trait, high oil plants, low oil plants, fine-scale mapping, *DGAT1-2* gene, triacylglycerol biosynthesis, chromosome 6.

Section 24.1

2. Summarize with a concept map the differences between continuous and discontinuous characteristics and the types of continuous traits. Include at least nine concepts in your map.

Section 24.3

3. Outline the concept of heritability by creating a concept map that includes the following terms: heritability, broad-sense heritability, additive genetic variance, narrow-sense heritability, phenotypic variance, genetic variance, genetic–environmental variance, elimination of variance components, parent–offspring regression, degrees of relatedness, gene interaction variance, dominance variance environmental variance.

Section 24.4

4. Create a concept map that summarizes the response to selection and how it is calculated, along with how genetic correlations affect the response to selection.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 25

Population Genetics

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Wolves on Isle Royale have been studied for over 50 years. Inbreeding and low genetic variation led to the population's decline.

The Wolves of Isle Royale

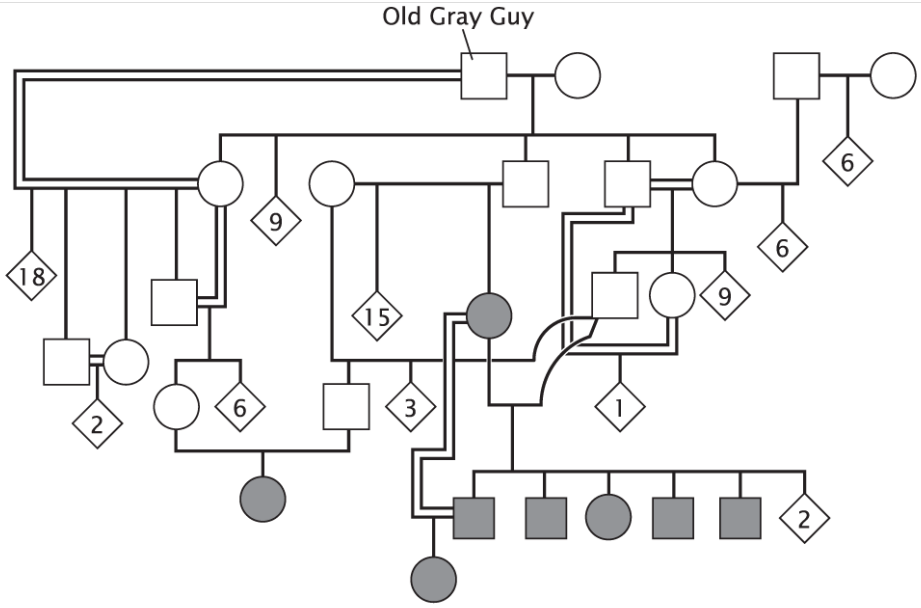
Rugged, isolated, and pristine, Isle Royale is surrounded by the deep, cold waters of northern Lake Superior. The island is a United States national park and wilderness area, covering only a little over 500 square kilometers of boreal forests and wetlands. Though small and

isolated, Isle Royale is famous for its wolves and is home to the longest-running study of predator–prey dynamics.

Moose first made it to Isle Royale in the early 1900s, most likely by swimming the 24 km of water that separates the island from the mainland. Because these moose had no natural predators, their numbers on Isle Royale mushroomed; the island's forest subsequently declined, as moose decimated the birch and aspen trees by browsing. This situation changed in 1949, when a single breeding pair of wolves trekked across the frozen lake in winter and set up residence on the island. The wolves preyed on the moose and kept their population in check. With fewer moose, the island's trees rebounded. When biologists initiated their study of moose–wolf interaction in 1959, there were 20 wolves and about 500 moose on the island.

Over the next 50 years, the numbers of wolves and moose on Isle Royale waxed and waned. In 1980, the wolf population reached an all-time high of 50 animals but crashed shortly afterward due to an epidemic of canine parvovirus introduced by a visiting domestic dog. After the epidemic, wolf numbers climbed from 14 to about 20 but remained low throughout the 1980s and early 1990s. The wolf population suffered from inbreeding and low genetic variation. As we will see in this chapter, inbreeding and the effects of chance factors operating in small populations (called genetic drift) lead to a loss of genetic variation, which often results in low fertility, birth defects, and lowered resistance to disease.

Then, in 1997, a remarkable event occurred. A lone male wolf, later dubbed Old Gray Guy for his light-colored fur, migrated across the winter ice to the island. He joined the Isle Royale wolves and introduced fresh genes that invigorated the population. Old Gray Guy was a prolific breeder. He eventually produced 34 offspring, and his genes came to dominate the population—by 2008, 59.4% of the wolf genes on the island were his. The population climbed to over 30 wolves, and moose numbers declined. Old Gray Guy died in 2006. Unfortunately, he had introduced a limited number of new genes. No more new wolves joined the population, and levels of inbreeding reached a new high (**Figure 25.1**). There was no reproduction in 2012, and by early 2013, only 8 wolves were left on the island. Their number dropped further in the following years—by 2016, only 2 wolves remained, a male and female pair. Genetic studies revealed that this pair was highly inbred: they were half siblings (born to the same mother), as well as father and daughter. Freed from wolf predation, the moose population boomed again.



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25.1 Pedigree of some of the wolves of Isle Royale. The shaded squares and circles represent wolves that were alive in 2012. Double lines represent matings between closely related individuals. [Data from P. W. Hedrick et al., Conservation Genetics 15: 1111 , 2014.]

Description

The pedigree shows an old gray guy mating with a female in P generation. They have two males, 1 offspring of unidentified sex numbered 9, and two female offspring. Another couple mates and has an offspring with unidentified sex numbered 6. Old gray guy mates with one of his female offspring and has 1 offspring with unidentified sex numbered 18, two male offspring and 1 female offspring in generation 2. The male of the other family from P generation mates with other female offspring of old gray guy and they have an offspring with unidentified sex numbered 6. A male offspring of old gray guy mates with a female and has 1 offspring with unidentified sex numbered 15 and a female who was alive in 2012. Another male offspring of old gray guy mates with old gray guy's female offspring and has a male, a female, and an offspring with unidentified sex numbered 9 in generation 3. A male and a female offspring of old gray guy with his female offspring mate and have an offspring of unidentified sex numbered 2 in generation 3. The female offspring of old gray guy who mated with him also mates their male offspring and they have a female offspring and an offspring with unidentified sex numbered 6 in generation 3. The mother of

unidentified sex offspring numbered 15 mates with a male sibling of unidentified offspring numbered 9 from generation 2 and they have a male and an offspring with unidentified sex numbered 3 in generation 3. The male offspring of old gray guy who mated with a female offspring of old gray guy mates with his female offspring and they have an offspring of unidentified sex numbered 1 in generation 3. A female offspring from generation 2 who was alive in 2012 mates with the male sibling of unidentified sex offspring numbered 9 from generation 2 and they have 4 males (all alive in 2012), 1 female (alive in 2012), and an offspring of unidentified sex numbered 2 in generation 4. The female offspring from generation 2 who was alive in 2012 also mates with one of his male offspring (alive in 2012) and has a female offspring in generation 5 (alive in 2012). The female sibling of unidentified sex offspring numbered 6 from generation 3 mates with male sibling of unidentified sex offspring numbered 3 from generation 3, and have a female offspring in generation 4 (alive in 2012).

Climate warming is one factor complicating the future of wolves on Isle Royale. Genetic studies suggest that Old Gray Guy was not the only wolf to migrate to the island since the population was established in 1949. Several wolves probably joined the population during its history. Migration is possible only during cold winters, when the lake waters freeze for several days to weeks at a time and wolves can walk from the mainland to the island. During the 1960s, ice bridges formed every few years, but recently they have formed much less frequently; climatologists project that Lake Superior will be largely ice free by 2040, cutting off all future wolf migration.

As the number of wolves on Isle Royale became precariously low, biologists proposed that additional wolves be transported to the island to counteract the effects of inbreeding. The introduction of new genetic variation into an inbred population, called **genetic rescue**, has been carried out for other inbred populations; it often

dramatically improves the health of such populations and can better ensure their long-term survival. Others felt that nature should be left to take its course, even if it led to the extinction of the Isle Royale wolf population.

After an extensive review, the National Park Service decided to introduce new genetic variation into the population. Four new wolves from Minnesota were broght to Isle Royale in 2018, and another seven wolves from Canada were added in early 2019. As of 2019, there were a total of 15 wolves on the island. The National Park Service plans to introduce up to 30 new wolves in to the population over the next several years.

THINK-PAIR-SHARE

- Imagine that you are asked to manage a population of wolves that have taken up residence on an island off the coast of Alaska. Because prey resources are limited, the island will support only about 50 wolves. What steps would you take to prevent inbreeding and genetic drift in the population in the future?
- Do you think the National Park Service was correct in bringing new wolves to Isle Royale in an attempt to rescue the population, or do you think that it should have allowed natural processes to proceed? Provide some possible reasons for and against attempting genetic rescue.

The wolves of Isle Royale illustrate several important principles of genetics: Small populations lose genetic variation over time through inbreeding and genetic drift, often with catastrophic consequences for survival and reproduction. Migration, whether occurring naturally or through genetic rescue, introduces new genetic

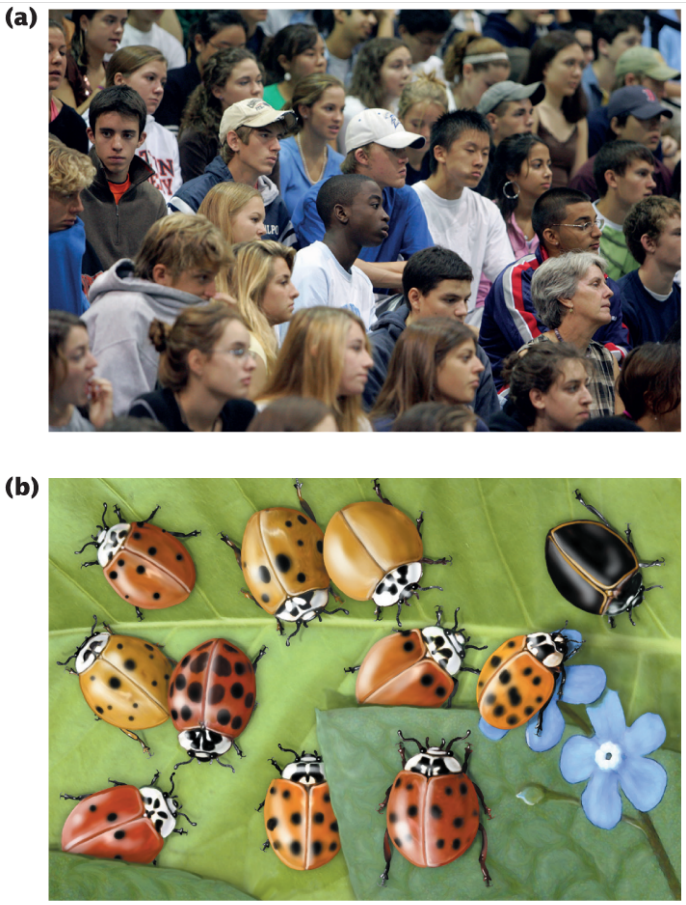
variation that counteracts the effects of genetic drift and inbreeding. These principles have important implications not only for wildlife management but also for the evolution of organisms in the natural world.

This chapter introduces *population genetics*, the branch of genetics that studies the genetic makeup of groups of individuals and how a group's genetic composition changes over time. Population geneticists usually focus their attention on a **Mendelian population**, which is a group of interbreeding, sexually reproducing individuals that have a common set of genes—the **gene pool**. A population evolves through changes in its gene pool; therefore, population genetics is also the study of evolution. Population geneticists study the variation in alleles within and between groups and the evolutionary forces responsible for shaping the patterns of genetic variation found in nature. In this chapter, we learn how the gene pool of a population is described and what factors are responsible for shaping it.

25.1 Genotypic and Allelic Frequencies Are Used to Describe the Gene Pool of a Population

An obvious and pervasive feature of life is variation. Students in a typical college class vary in eye color, hair color, skin pigmentation, height, weight, facial features, blood type, and susceptibility to numerous diseases and disorders. No two students in the class are likely to be the same in appearance.

Humans are not unique in their extensive variation (**Figure 25.2a**); almost all organisms exhibit phenotypic variation. For instance, lady beetles are highly variable in their spotting patterns (**Figure 25.2b**), mice vary in color, snails have different numbers of stripes on their shells, and plants vary in their susceptibility to pests. Much of this phenotypic variation is hereditary. Recognition of the extent of phenotypic variation led Charles Darwin to the idea of evolution through natural selection. Genetic variation is the basis of all evolution, and the extent of genetic variation within a population affects its potential to adapt to environmental change.



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Part a: Michael Dwyer/Alamy.

25.2 All organisms exhibit genetic variation. (a) Extensive variation among humans. (b) Variation in the spotting patterns of Asian lady beetles.

Description
Photo a shows a diverse crowd of people sitting in a stadium. Photo b shows Asian lady beetles on a leaf. All the beetles have different patterns of spotting with different base colors while some don't have any spots.

In fact, even more genetic variation exists in populations than is visible in the phenotype. A great deal of variation exists at the molecular level, owing in part to the redundancy of the genetic code,

which allows different codons to specify the same amino acid. Thus, two members of a population can produce the same protein, even if their DNA sequences are different. There is also variation in DNA sequences between genes and introns within genes, which do not encode proteins; some of this variation is thought to have little effect on the phenotype. Although this variation may not affect the phenotype, it is often useful for determining evolutionary relationships among organisms and understanding the evolutionary forces that have shaped a species.

Mathematical Models for Understanding Genetic Variation

An important but frequently misunderstood tool used for investigating the nature of genetic variation in populations is the mathematical model. Let's take a moment to consider what a model is and how it can be used. A mathematical model usually describes a process as an equation. Factors that may influence the process are represented by variables in the equation; the equation defines the way in which the variables influence the process. Most models are simplified representations of a process because simultaneous consideration of all the factors involved is impossible; some factors must be ignored in order to examine the effects of others. At first, a model might consider only one or a few factors, but after their effects are understood, the model can be improved by the addition

of more details. Even a simple model, however, can be a source of valuable insight into how a process is influenced by key variables.

Before we can explore the evolutionary forces that shape genetic variation, we must be able to describe the genetic structure of a population. The usual way of describing this structure is to enumerate the types and frequencies of genotypes and alleles in a population. A frequency is simply a proportion or a percentage, usually expressed as a decimal fraction. For example, if 20% of the alleles at a particular locus in a population are *A*, we would say that the frequency of the *A* allele in the population is 0.20. For large populations, for which a determination of the genotypes of all individual members is impractical, a sample of the population is usually taken (see Section 24.2) and the genotypic and allelic frequencies are calculated for this sample. The genotypic and allelic frequencies of the sample are then used to represent the gene pool of the population.

Calculating Genotypic Frequencies

To calculate a **genotypic frequency**, we simply add up the number of individuals possessing a genotype and divide by the total number of individuals in the sample (*N*). For a locus with three genotypes—, *AA*, *Aa*, and *aa*— the frequency (*f*) of each genotype is as follows:

$$
\begin{aligned}
f(AA) &= \frac{\text{number of } AA \text{ individuals}}{N} \\
f(Aa) &= \frac{\text{number of } Aa \text{ individuals}}{N} \\
f(aa) &= \frac{\text{number of } aa \text{ individuals}}{N}
\end{aligned}
\tag{25.1}
$$

The sum of all the genotypic frequencies always equals 1.

Calculating Allelic Frequencies

The gene pool of a population can also be described in terms of **allelic frequencies**. There are always fewer alleles than genotypes, so the gene pool of a population can be described in fewer terms when allelic frequencies are used. In a sexually reproducing population, the genotypes are only temporary assemblages of the alleles. As described by Mendel's principle of segregation, individual alleles, not genotypes, are passed from generation to generation through the gametes, and genotypes re-form from alleles in each generation. Thus, the types and numbers of alleles, rather than genotypes, have real continuity from one generation to the next and make up the gene pool of a population.

Allelic frequencies can be calculated from (1) the numbers or (2) the frequencies of the genotypes. To calculate the allelic frequency from

the numbers of the genotypes, we count the number of copies of a particular allele present at a locus in a sample and divide by the total number of all alleles in the sample:

$$\text{frequency of an allele } = \frac{\begin{array}{c}\text{number of copies} \\ \text{of the allele}\end{array}}{\begin{array}{c}\text{number of copies of all} \\ \text{alleles at the locus}\end{array}} \tag{25.2}$$

For a locus with only two alleles (*A* and *a*), the frequencies of the alleles are usually represented by the symbols *p* and *q*. The frequencies can be calculated as follows:

$$\begin{aligned} p = f(A) &= \frac{2n_{AA} + n_{Aa}}{2N} \\ q = f(a) &= \frac{2n_{aa} + n_{Aa}}{2N} \end{aligned} \tag{25.3}$$

where n_{AA}, n_{Aa}, and n_{aa} represent the numbers of *AA*, *Aa*, and *aa* individuals, and *N* represents the total number of individuals in the sample. To obtain the number of copies of the allele in the numerator of the equation, we add twice the number of homozygotes (because each has two copies of the allele for which the frequency is being calculated) to the number of heterozygotes (because each has a single copy of the allele). We divide by $2N$ because each diploid individual has two alleles at a locus. The sum

of the allelic frequencies always equals 1($p + q = 1$); after p has been obtained, q can be determined by subtraction: $q = 1 - p$.

Alternatively, allelic frequencies can be calculated from the genotypic frequencies. This method is useful if the genotypic frequencies have already been calculated and the numbers of the different genotypes are not available. To calculate an allelic frequency from genotypic frequencies, we add the frequency of the homozygote for each allele to half the frequency of the heterozygote (because half of the heterozygote's alleles are of each type):

$$p = f(A) = f(AA) + {}^{1}\!/_{2}\ f(Aa)$$
$$q = f(a) = f(aa) + {}^{1}\!/_{2}\ f(Aa) \tag{25.4}$$

We obtain the same values of p and q whether we calculate the allelic frequencies from the numbers of the genotypes (Equation 25.3) or from the genotypic frequencies (Equation 25.4). A sample calculation of allelic frequencies is provided in the next Worked Problem. › **TRY PROBLEM 16**

LOCI WITH MULTIPLE ALLELES

We can use the same principles to determine the frequencies of alleles for loci with more than two alleles. To calculate the allelic

frequencies from the numbers of genotypes, we count up the number of copies of an allele by adding twice the number of homozygotes to the number of heterozygotes that possess the allele, then dividing this sum by twice the number of individuals in the sample. For a locus with three alleles (A^1, A^2, and A^3) and six genotypes (A^1A^1, A^1A^2, A^2A^2, A^1A^3, A^2A^3, and A^3A^3), the frequencies (p, q, and r) of the alleles are as follows:

$$
\begin{aligned}
p = f(A^1) &= \frac{2n_{A^1A^1} + n_{A^1A^2} + n_{A^1A^3}}{2N} \\
q = f(A^2) &= \frac{2n_{A^2A^2} + n_{A^1A^2} + n_{A^2A^3}}{2N} \\
r = f(A^3) &= \frac{2n_{A^3A^3} + n_{A^1A^3} + n_{A^2A^3}}{2N}
\end{aligned}
\tag{25.5}
$$

Alternatively, we can calculate the frequencies of multiple alleles from the genotypic frequencies by extending Equation 25.4. Once again, we add the frequency of the homozygote to half the frequency of each heterozygous genotype that possesses the allele:

$$
\begin{aligned}
p &= f(A^1A^1) + {}^1\!/_2 f(A^1A^2) + {}^1\!/_2 f(A^1A^3) \\
q &= f(A^2A^2) + {}^1\!/_2 f(A^1A^2) + {}^1\!/_2 f(A^2A^3) \\
r &= f(A^3A^3) + {}^1\!/_2 f(A^1A^3) + {}^1\!/_2 f(A^2A^3)
\end{aligned}
\tag{25.6}
$$

X-LINKED LOCI

To calculate allelic frequencies for genes at X-linked loci, we apply these same principles. However, we must remember that a female possesses two X chromosomes and therefore has two X-linked alleles, whereas a male has only a single X chromosome and one X-linked allele.

Suppose there are two alleles at an X-linked locus, X^A and X^a. Females may be either homozygous ($\mathrm{X}^A\mathrm{X}^A$ or $\mathrm{X}^a\mathrm{X}^a$) or heterozygous ($\mathrm{X}^A\mathrm{X}^a$). All males are hemizygous ($\mathrm{X}^A\mathrm{Y}$ or $\mathrm{X}^a\mathrm{Y}$). To determine the frequency of the X^A allele (p), we first count the number of copies of X^A: we multiply the number of $\mathrm{X}^A\mathrm{X}^A$ females by two and add the number of $\mathrm{X}^A\mathrm{X}^a$ females and the number of $\mathrm{X}^A\mathrm{Y}$ males. We then divide the sum by the total number of alleles at the locus, which is twice the total number of females plus the number of males:

$$p = f(X^A) = \frac{2n_{\mathrm{X}^A\mathrm{X}^A} + n_{\mathrm{X}^A\mathrm{X}^a} + n_{\mathrm{X}^A\mathrm{Y}}}{2n_{\text{females}} + n_{\text{females}}} \tag{25.7a}$$

Similarly, the frequency of the X^a allele is

$$q = f(X^a) = \frac{2n_{\mathrm{X}^a\mathrm{X}^a} + n_{\mathrm{X}^A\mathrm{X}^a} + n_{\mathrm{X}^a\mathrm{Y}}}{2n_{\text{females}} + n_{\text{females}}} \tag{25.7b}$$

The frequencies of X-linked alleles can also be calculated from genotypic frequencies by adding the frequency of the females that

are homozygous for the allele, half the frequency of the females that are heterozygous for the allele, and the frequency of males that are hemizygous for the allele:

$$p = f(\mathrm{X}^A) = f(\mathrm{X}^A\mathrm{X}^A) + {}^1\!/_2 f(\mathrm{X}^A\mathrm{X}^a) + f(\mathrm{X}^A\mathrm{Y})$$
$$q = f(\mathrm{X}^a) = f(\mathrm{X}^a\mathrm{X}^a) + {}^1\!/_2 f(\mathrm{X}^A\mathrm{X}^a) + f(\mathrm{X}^a\mathrm{Y})$$

(25.8)

If you remember the logic behind these calculations, you can determine allelic frequencies for any set of genotypes, and it will not be necessary to memorize all the formulas. ❯ **TRY** PROBLEM 18

CONCEPTS

Population genetics concerns the genetic composition of a population and how it changes over time. The gene pool of a population can be described by the frequencies of genotypes and alleles in the population.

✔ CONCEPT CHECK 1

What are some advantages of using allelic frequencies to describe the gene pool of a population over using genotypic frequencies?

WORKED PROBLEM

The human MN blood-type antigens are determined by two codominant alleles, L^M and L^N (see p. 114 in Chapter 5). The MN blood types and corresponding genotypes of 398 Finns from Karjala are tabulated here:

Phenotype	Genotype	Number
M	L^ML^M	182
MN	L^ML^N	172
N	L^NL^N	44

Source: Data from W. C. Boyd, *Genetics and the Races of Man*. Boston: Little, Brown, 1950.

Calculate the genotypic and allelic frequencies at the MN locus for the Karjala population.

Solution Strategy

What information is required in your answer to the problem?

The genotypic and allelic frequencies of the population.

What information is provided to solve the problem?

The numbers of the different MN genotypes in the sample.

Solution Steps

The genotypic frequencies for the population are calculated with the following formula:

$$\begin{aligned}
\text{genotypic frequency} &= \frac{\text{number of individuals with genotype}}{\text{total number of individuals in sample } (N)} \\
f(L^M L^M) &= \frac{\text{number of } L^M L^M \text{ individuals}}{N} = \frac{182}{398} \\
f(L^M L^N) &= \frac{\text{number of } L^M L^N \text{ individuals}}{N} = \frac{172}{398} \\
f(L^N L^N) &= \frac{\text{number of } L^N L^N \text{ individuals}}{N} = \frac{44}{398}
\end{aligned}$$

The genotypic frequencies sum to 1.0. The allelic frequencies can be calculated from either the numbers or the frequencies of the genotypes. To calculate allelic frequencies from numbers of genotypes, we count the number of copies of each allele and divide by the number of copies of all alleles at that locus:

$$\begin{aligned}
\text{frequency of an allele} &= \frac{\text{number of copies of the allele}}{\text{number of copies of all alleles}} \\
p &= f(L^M) = \frac{(2n_{L^{\mathrm{M}}L^{\mathrm{M}}}) + (n_{L^{\mathrm{M}}L^{\mathrm{N}}})}{2N} \\
&= \frac{2(182) + 172}{2(398)} = \frac{536}{796} = 0.673 \\
q &= f(L^N) = \frac{(2n_{L^{\mathrm{N}}L^{\mathrm{N}}}) + (n_{L^{\mathrm{M}}L^{\mathrm{N}}})}{2N} \\
&= \frac{2(44) + 172}{2(398)} = \frac{260}{796} = 0.327
\end{aligned}$$

To calculate the allelic frequencies from genotypic frequencies, we add the frequency of the homozygote for that genotype to half the frequency of each heterozygote possessing that allele:

$$\begin{aligned} p &= f\left(L^{\mathrm{M}}\right) = f\left(L^{\mathrm{M}}L^{\mathrm{M}}\right) + {}^{1}\!/_{2} f\left(L^{\mathrm{M}}L^{\mathrm{N}}\right) \\ &= 0.457 + {}^{1}\!/_{2}(0.432) = 0.673 \\ q &= f\left(L^{\mathrm{N}}\right) = f\left(L^{\mathrm{N}}L^{\mathrm{N}}\right) + {}^{1}\!/_{2} f\left(L^{\mathrm{M}}L^{\mathrm{N}}\right) \\ &= 0.111 + {}^{1}\!/_{2}(0.432) = 0.327 \end{aligned}$$

» Now try your hand at calculating genotypic and allelic frequencies by working **Problem 17** at the end of the chapter.

25.2 The Hardy–Weinberg Law Describes the Effect of Reproduction on Genotypic and Allelic Frequencies

The primary goal of population genetics is to understand the processes that shape a population's gene pool. First, we must ask what effects reproduction and Mendelian principles have on the genotypic and allelic frequencies. How do the segregation of alleles in gamete formation and the combining of alleles in fertilization influence the gene pool? The answer to this question lies in the **Hardy–Weinberg law**, among the most important principles of population genetics.

The Hardy–Weinberg law was formulated independently by G. H. Hardy and Wilhelm Weinberg in 1908 (similar conclusions were reached by several other geneticists at about the same time). The law is actually a mathematical model that evaluates the effect of reproduction on the genotypic and allelic frequencies of a population. It makes several simplifying assumptions about the population and provides two key predictions if these assumptions are met. For an autosomal locus with two alleles, the Hardy–Weinberg law can be stated as follows:

> **Assumptions** If a population is large; randomly mating; and not affected by mutation, migration, or natural selection, then

Prediction 1 the allelic frequencies of a population do not change, and
Prediction 2 the genotypic frequencies stabilize (will not change) after one generation in the proportions p^2 (the frequency of *AA*), $2pq$ (the frequency of *Aa*), and q^2 (the frequency of *aa*), where p equals the frequency of allele *A* and q equals the frequency of allele *a*.

The Hardy–Weinberg law indicates that when its assumptions are met, reproduction alone does not alter allelic or genotypic frequencies, and the allelic frequencies determine the frequencies of genotypes.

The statement that the genotypic frequencies stabilize after one generation means that they may change after the first generation, because one generation of random mating is required to produce Hardy–Weinberg proportions of the genotypes. Afterward, the genotypic frequencies, like the allelic frequencies, do not change as long as the population continues to meet the assumptions of the Hardy–Weinberg law. When genotypes are in the expected proportions of p^2, $2pq$, and q^2, the population is said to be in **Hardy–Weinberg equilibrium**.

CONCEPTS

The Hardy–Weinberg law describes how reproduction and Mendelian principles affect the allelic and genotypic frequencies of a population.

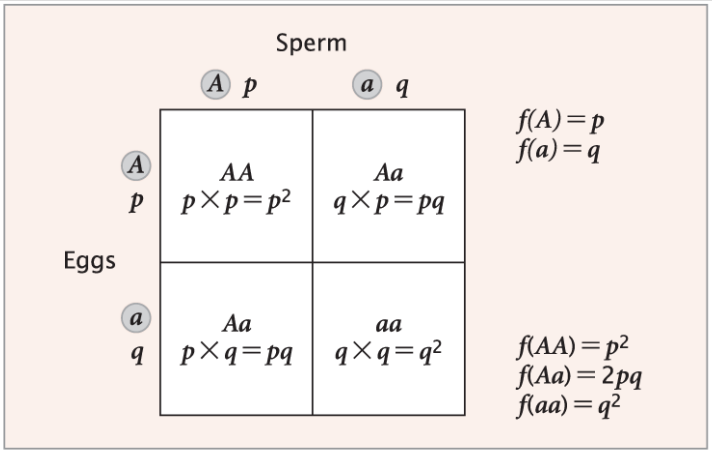
✔ CONCEPT CHECK 2

Which of the following statements is not an assumption of the Hardy–Weinberg law?

a. The allelic frequencies (p and q) are equal.
b. The population is randomly mating.
c. The population is large.
d. Natural selection has no effect.

Genotypic Frequencies at Hardy–Weinberg Equilibrium

How do the assumptions of the Hardy–Weinberg law lead to genotypic proportions of p^2, $2pq$, and q^2? Mendel's principle of segregation says that each individual organism possesses two alleles at a locus and that each of those two alleles has an equal probability of passing into a gamete. Thus, the frequencies of alleles in gametes will be the same as the frequencies of alleles in the parents. Suppose that we have a Mendelian population in which the frequencies of alleles A and a are p and q, respectively. These frequencies will also be those in the gametes. If mating is random (one of the assumptions of the Hardy–Weinberg law), the gametes will come together in random combinations, which can be represented by a Punnett square (**Figure 25.3**).



25.3 Random mating produces genotypes in the proportions p^2, $2pq$, and q^2.

Note that this square represents mating in a population, not an individual cross.

Description

The Punnett square has 2 rows and 2 columns. The rows represent eggs and the columns represent sperm. The column headers are as follows from left to right: big A (shaded) small P and small A (shaded) small Q. The row headers are as follows from top to bottom: big A (shaded) small P and small A (shaded) small Q.

The row entries are as follows:

Row 1, column 1: big A big A, p multiplied by p equals p squared.

Row 1, column 2: big A small A, q multiplied by p equals p q.

Row 2, column 1: big A small A, p multiplied by q equals p q.

Row 2, column 2: small A small A, q multiplied by q equals q squared.

Text at the top right reads, f of open parenthesis big A close parenthesis equals p. f of open parenthesis small A close parenthesis equals q.

Text at the bottom right reads, f of open parenthesis big A big A close parenthesis equals p squared. f of open parenthesis big A small A close parenthesis equals 2 p q. f of open

parenthesis small A small A close parenthesis equals q squared.

A caption at the bottom reads, Conclusion: Random mating will produce genotypes of the next generation in proportions p squared open parenthesis big A big A close parenthesis, 2 p q open parenthesis big A small A close parenthesis, and q squared open parenthesis small A small A close parenthesis.

The multiplication rule (see Section 3.2) can be used to determine the probability of various gametes pairing. For example, the probability of a sperm containing allele A is p and the probability of an egg containing allele A is p. Applying the multiplication rule, we find that the probability that these two gametes will combine to produce an AA homozygote is $p \times p = p^2$. Similarly, the probability of a sperm containing allele a combining with an egg containing allele a to produce an aa homozygote is $q \times q = q^2$. An Aa heterozygote can be produced in one of two ways: (1) a sperm containing allele A may combine with an egg containing allele a ($p \times q$) or (2) an egg containing allele A may combine with a sperm containing allele a ($p \times q$). Thus, the probability of alleles A and a combining to produce an Aa heterozygote is $2pq$. In summary, whenever the frequencies of alleles in a randomly mating population are p and q, the frequencies of the genotypes in the next generation will be p^2, $2pq$, and q^2. Figure 25.3 demonstrates that only a single generation of random mating is required to produce the Hardy–Weinberg genotypic proportions.

Closer Examination of the Hardy–Weinberg Law

Before we consider the implications of the Hardy–Weinberg law, we need to take a closer look at the three assumptions that it makes about a population. First, it assumes that the population is large. How big is "large"? Theoretically, the Hardy–Weinberg law requires that a population be infinitely large in size, but this requirement is obviously unrealistic. In practice, many large populations have genotypes in the predicted Hardy–Weinberg proportions, and significant deviations arise only when population size is rather small. In Section 25.4, we will examine the effects of small population size on allelic frequencies.

The second assumption of the Hardy–Weinberg law is that members of the population mate randomly with respect to genotype, which means that each genotype mates relative to its frequency. For example, suppose that three genotypes are present in a population in the following proportions: $f(AA) = 0.6$, $f(Aa) = 0.3$, and $f(aa) = 0.1$. With random mating, the frequency of mating between two AA homozygotes ($AA \times AA$) will be equal to the product of their frequencies: $0.6 \times 0.6 = 0.36$, whereas the frequency of mating between two aa homozygotes ($aa \times aa$) will be only $0.1 \times 0.1 = 0.01$.

The third assumption of the Hardy–Weinberg law is that the allelic frequencies of the population are not affected by natural selection,

migration, or mutation. Although mutation occurs in every population, its rate is so low that it has little short-term effect on the predictions of the Hardy–Weinberg law (although it may shape allelic frequencies over long periods when no other forces are acting). Although natural selection and migration are significant factors in real populations, we must remember that the purpose of the Hardy–Weinberg law is to examine only the effect of reproduction on the gene pool. When this effect is known, the effects of other factors (such as migration and natural selection) can be examined.

A final point is that the assumptions of the Hardy–Weinberg law apply to a single locus. No real population mates randomly for all traits, and no population is completely free of natural selection for all traits. The Hardy–Weinberg law, however, does not require random mating and the absence of selection, migration, and mutation for all traits; it requires these conditions only for the locus under consideration. A population may be in Hardy–Weinberg equilibrium for one locus but not for others.

Implications of the Hardy–Weinberg Law

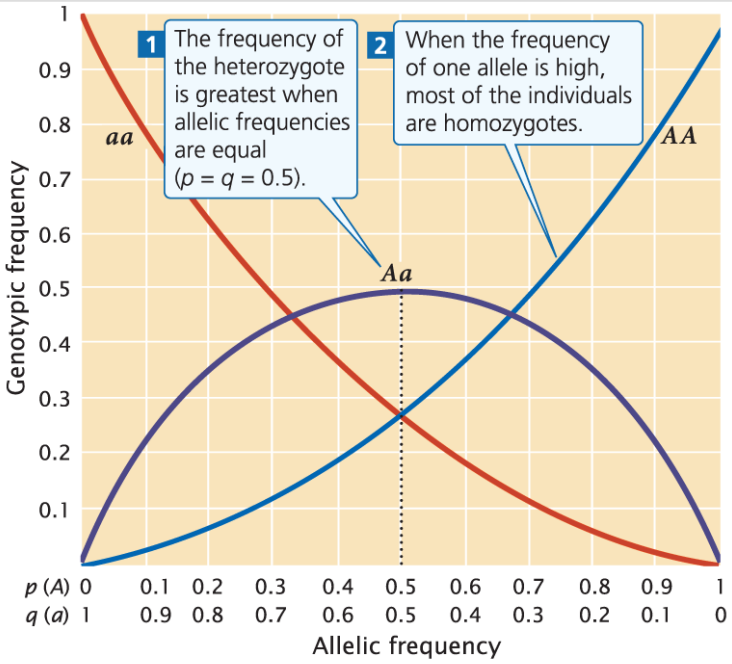
The Hardy–Weinberg law has several important implications for the genetic structure of a population. One implication is that a

population cannot evolve if it meets the Hardy–Weinberg assumptions, because evolution consists of change in the allelic frequencies of a population. Therefore, the Hardy–Weinberg law tells us that reproduction alone will not bring about evolution. Other processes—such as mutation, migration, and natural selection—or chance events are required for populations to evolve.

A second important implication is that when a population is in Hardy–Weinberg equilibrium, the genotypic frequencies are determined by the allelic frequencies. When a population is not in Hardy–Weinberg equilibrium, we have no basis for predicting the genotypic frequencies. Although we can always determine the allelic frequencies from the genotypic frequencies (see Equation 25.3), the reverse (determining the genotypic frequencies from the allelic frequencies) is possible only when the population is in Hardy–Weinberg equilibrium.

For a locus with two alleles, the frequency of the heterozygote is greatest when allelic frequencies are between 0.33 and 0.66 and is at a maximum when allelic frequencies are each 0.5 (**Figure 25.4**). The heterozygote frequency never exceeds 0.5 when the population is in Hardy–Weinberg equilibrium. Furthermore, when the frequency of one allele is low, homozygotes for that allele will be rare, and most of the copies of a rare allele will be present in heterozygotes. As you can see from Figure 25.4, when the frequency of allele *a* is 0.2, the frequency of *aa* homozygotes is only 0.04 (q^2), but the frequency of *Aa* heterozygotes is 0.32 ($2pq$); 80% of the *a* alleles are in

heterozygotes. Use (A) **Animation 25.1** to examine the effect of allelic frequencies on genotypic frequencies when a population is in Hardy–Weinberg equilibrium.



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020
W. H. Freeman and Company

25.4 When a population is in Hardy–Weinberg equilibrium, the proportions of genotypes are determined by the frequencies of alleles.

Description

The horizontal axis represents allelic frequency. It shows ranges: p open parenthesis big A close parenthesis ranging from 0 to 1, in increments of 0 point 1. q open parenthesis big A close parenthesis ranging from 1 to 0, in increments of negative 0 point 1. The vertical axis represents genotypic frequency and ranges from 0 point 1 to 1, in increments of 0 point 1. A semi circular curve representing big A small A starts from (0, 0), peaks at (0 point 5, 0 point 5), and ends at (1, 0). A caption reads, The frequency of the heterozygote is greatest when allelic frequencies are equal (p equals q equals 0 point 5). A concave up increasing curve representing big A big A begins from (0, 0) and ends at (1, 0 point 9 7). A caption reads, When the frequency of one allele is high, most of the individuals are homozygotes. A concave up decreasing curve representing small A small A starts at (0, 1) and ends at

(1, 0). A dotted vertical line begins from (0 point 5, 0) and intersects concave up curves at (0 point 5, 0 point 2 8), then finally touches the semicircular curve at (0 point 5, 0 point 5). All values are approximate.

A third implication of the Hardy–Weinberg law is that a single generation of random mating produces the equilibrium frequencies of p^2, $2pq$, and q^2. The fact that genotypes are in Hardy–Weinberg proportions does not prove that the population is free from natural selection, mutation, and migration. It means only that these forces have not acted since the last time random mating took place.

A final implication is that when a population is not in Hardy–Weinberg equilibrium, one of the assumptions of the law has not been met, although without further investigation it will not be apparent which assumption has been violated. Finding that a population is not in equilibrium often leads to other studies to determine what evolutionary forces are acting on the population.

Extensions of the Hardy–Weinberg Law

The Hardy–Weinberg expected proportions can also be applied to multiple alleles and X-linked alleles (Table 25.1). With multiple alleles, the genotypic frequencies expected at equilibrium are the square of the allelic frequencies. For an autosomal locus with three alleles, the equilibrium genotypic frequencies are $(p + q + r)^2 = p^2 +$

$2pq + q^2 + 2pr + 2qr + r^2$. For an X-linked locus with two alleles, X^1 and X^2, the equilibrium frequencies of the female genotypes are $(p + q)^2 = p^2 + 2pq + q^2$, where p^2 is the frequency of X^1X^1, $2pq$ is the frequency of X^1X^2, and q^2 is the frequency of X^2X^2. Males have only a single X-linked allele, so the frequencies of the male genotypes are p (frequency of X^1Y) and q (frequency of X^2Y). These proportions are those of the genotypes among males and among females, rather than the proportions among the entire population. Thus, p^2 is the expected proportion of females with the genotype X^1X^1; if females make up 50% of the population, then the expected proportion of this genotype in the entire population is $0.5 \times p^2$.

TABLE 25.1 Extensions of the Hardy–Weinberg law

Situation	Allelic Frequencies	Genotypic Frequencies
Three alleles	$f(A^1) = p$	$f(A^1A^1) = p^2$
	$f(A^2) = q$	$f(A^1A^2) = 2pq$
	$f(A^3) = r$	$f(A^2A^2) = q^2$
		$f(A^1A^3) = 2pr$
		$f(A^2A^3) = 2qr$
		$f(A^3A^3) = r^2$

X-linked alleles	$f(X^1) = p$	$f(X^1X^1 \text{ female}) = p^2$
	$f(X^2) = q$	$f(X^1X^2 \text{ female}) = 2pq$
		$f(X^2X^2 \text{ female}) = q^2$
		$f(X^1Y \text{ male}) = p$
		$f(X^2Y \text{ male}) = q$

For X-linked female genotypes, the frequencies are the proportions among all females; for X-linked male genotypes, the frequencies are the proportions among all males.

Notice that the frequency of an X-linked recessive trait among males is q, whereas the frequency among females is q^2. When an X-linked allele is uncommon, the trait will therefore be much more frequent in males than in females. Consider hemophilia A, a blood-clotting disorder caused by an X-linked recessive allele with a frequency (q) of approximately 1 in 10,000, or 0.0001. At Hardy–Weinberg equilibrium, this frequency will also be the frequency of the disease among males. The frequency of the disease among females, however, will be $q^2 = (0.0001)^2 = 0.00000001$, which is only 1 in 100 million. Hemophilia is 10,000 times more frequent in males than in females.

Testing for Hardy–Weinberg Proportions

To determine whether a population's genotypes are in Hardy–Weinberg equilibrium, the genotypic frequencies expected under the Hardy–Weinberg law must be compared with the observed genotypic frequencies. To do this, we first calculate the allelic frequencies, then find the expected genotypic frequencies by using the square of the allelic frequencies, and finally compare the observed and expected genotypic frequencies by using a chi-square goodness-of-fit test.

WORKED PROBLEM

Jeffrey Mitton and his colleagues found three genotypes (R^2R^2, R^2R^3, and R^3R^3) at a locus encoding the enzyme peroxidase in ponderosa pine trees growing at Glacier Lake, Colorado. The observed numbers of these genotypes are given in the following table.

Genotypes	Number observed
R^2R^2	135
R^2R^3	44
R^3R^3	11

Are the ponderosa pine trees at Glacier Lake in Hardy–Weinberg equilibrium at the peroxidase locus?

Solution Strategy

What information is required in your answer to the problem?

The results of a chi-square test, to determine whether the population is in Hardy–Weinberg equilibrium.

What information is provided to solve the problem?

The numbers of the different genotypes in a sample of the population.

Solution Steps

If the frequency of the R^2 allele equals p and the frequency of the R^3 allele equals q, the frequency of the R^2 allele is

$$p = f(R^2) = \frac{(2n_{R^2R^2}) + (n_{R^2R^3})}{2N} = \frac{2(135) + 44}{2(190)} = 0.826$$

The frequency of the R^3 allele is obtained by subtraction:

$$q = f(R^3) = 1 - p = 0.174$$

The frequencies of the genotypes expected under Hardy–Weinberg equilibrium are then calculated using p^2, $2pq$, and q^2:

$$
\begin{aligned}
R^2R^2 &= p^2 = (0.826)^2 = 0.683 \\
R^2R^3 &= 2pq = 2(0.826)(0.174) = 0.287 \\
R^3R^3 &= q^2 = (0.174)^2 = 0.03
\end{aligned}
$$

Multiplying each of these expected genotypic frequencies by the total number of observed genotypes in the sample (190), we obtain the numbers expected for each genotype:

$$
\begin{aligned}
R^2R^2 &= 0.683 \times 190 = 129.8 \\
R^2R^3 &= 0.287 \times 190 = 54.5 \\
R^3R^3 &= 0.03 \times 190 = 5.7
\end{aligned}
$$

By comparing these expected numbers with the observed numbers of each genotype, we see that there are more R^2R^2 and R^3R^3 homozygotes and fewer R^2R^3 heterozygotes in the population than we expect at equilibrium.

A chi-square goodness-of-fit test (see Section 3.4) is used to determine whether the differences between the observed and the expected numbers of each genotype are due to chance:

$$
\begin{aligned}
\chi^2 &= \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}} \\
&= \frac{(135 - 129.8)^2}{129.8} + \frac{(44 - 54.5)^2}{54.5} + \frac{(11 - 5.7)^2}{5.7} \\
&= 0.21 + 2.02 + 4.93 = 7.16
\end{aligned}
$$

The calculated chi-square value is 7.16. To obtain the probability associated with this chi-square value, we determine the appropriate degrees of freedom.

Up to this point, the chi-square test for assessing Hardy–Weinberg equilibrium has been identical with the chi-square tests that we used in Chapter 3 to assess progeny ratios in a genetic cross, in which the degrees of freedom were $n - 1$, and n equaled the number of expected genotypes. For the Hardy–Weinberg test, however, we must subtract an additional degree of freedom, because the expected numbers are based on the observed allelic frequencies; therefore, the observed numbers are not completely free to vary. In general, the degrees of freedom for a chi-square test of Hardy–Weinberg equilibrium equal the number of expected genotypic classes minus the number of associated alleles. For this particular Hardy–Weinberg test, the degrees of freedom are $3 - 2 = 1$.

After we have calculated both the chi-square value and the degrees of freedom, the probability associated with this value can be sought in a chi-square table (see Table 3.7). With 1 degree of freedom, a chi-square value of 7.16 has a probability between 0.01 and 0.001. It is very unlikely that the peroxidase genotypes observed at Glacier Lake are in Hardy–Weinberg equilibrium.

» For additional practice, determine whether the genotypic frequencies in **Problem 23** at the end of the chapter are in Hardy–Weinberg equilibrium.

CONCEPTS

The observed number of genotypes in a population can be compared with the Hardy–Weinberg expected proportions by using a chi-square goodness-of-fit test.

✔ CONCEPT CHECK 3

What is the expected frequency of heterozygotes in a population with allelic frequencies *x* and *y* that is in Hardy–Weinberg equilibrium?

a. $x + y$
b. xy
c. $2xy$
d. $(x - y)^2$

Estimating Allelic Frequencies with the Hardy–Weinberg Law

A practical use of the Hardy–Weinberg law is that it allows us to calculate allelic frequencies when dominance is present. For example, cystic fibrosis is a life-threatening autosomal recessive disorder characterized by frequent and severe respiratory infections, incomplete digestion, and abnormal sweating (see Section 5.1). Among North American Caucasians, the incidence of the disease is approximately 1 person in 2000. The formula for calculating allelic frequencies (see Equation 25.3) requires that we know the numbers of homozygotes and heterozygotes, but cystic fibrosis is a recessive disease, so we cannot easily distinguish between homozygous

unaffected persons and heterozygous carriers. Although molecular tests are available for identifying heterozygous carriers of the cystic fibrosis gene, the low frequency of the disease makes widespread screening impractical. In such situations, the Hardy–Weinberg law can be used to estimate the allelic frequencies.

If we assume that a population is in Hardy–Weinberg equilibrium with regard to this locus, then the frequency of the recessive genotype (*aa*) is q^2, and the frequency of the recessive allele is the square root of that genotypic frequency:

$$q = \sqrt{f(aa)} \tag{25.9}$$

If the frequency of cystic fibrosis in North American Caucasians is approximately 1 in 2000, or 0.0005, then $q = \sqrt{0.0005} = 0.02$. Thus, about 2% of the alleles in the Caucasian population encode the defective protein that causes cystic fibrosis. We can calculate the frequency of the normal allele by subtracting: $p = 1 - q = 1 - 0.02 = 0.98$. After we have calculated p and q, we can use the Hardy–Weinberg law to determine the frequencies of homozygous unaffected people and heterozygous carriers of the cystic fibrosis allele:

$$\begin{aligned} f(AA) &= p^2 = (0.98)^2 = 0.960 \\ f\,(Aa) &= 2pq = 2(0.02)(0.98) = 0.0392 \end{aligned}$$

Thus, about 4% of Caucasians (1 in 25) are heterozygous carriers of the allele that causes cystic fibrosis. ❯ **TRY** **PROBLEM 26**

THINK-PAIR-SHARE **Question 1**

CONCEPTS

Although allelic frequencies cannot be calculated directly for a locus that exhibits dominance, the Hardy–Weinberg law can be used to estimate allelic frequencies if the population is in Hardy–Weinberg equilibrium for that locus. The frequency of the recessive allele is equal to the square root of the frequency of the recessive trait.

✔ CONCEPT CHECK 4

In cats, all-white color is dominant over colors other than all-white. In a population of 100 cats, 19 are all-white. Assuming that the population is in Hardy–Weinberg equilibrium, what is the frequency of the all-white allele in this population?

25.3 Nonrandom Mating Affects the Genotypic Frequencies of a Population

An assumption of the Hardy–Weinberg law is that mating is random with respect to genotype. Although it does not alter the frequencies of alleles, nonrandom mating affects the way in which alleles combine to form genotypes and alters the genotypic frequencies of a population.

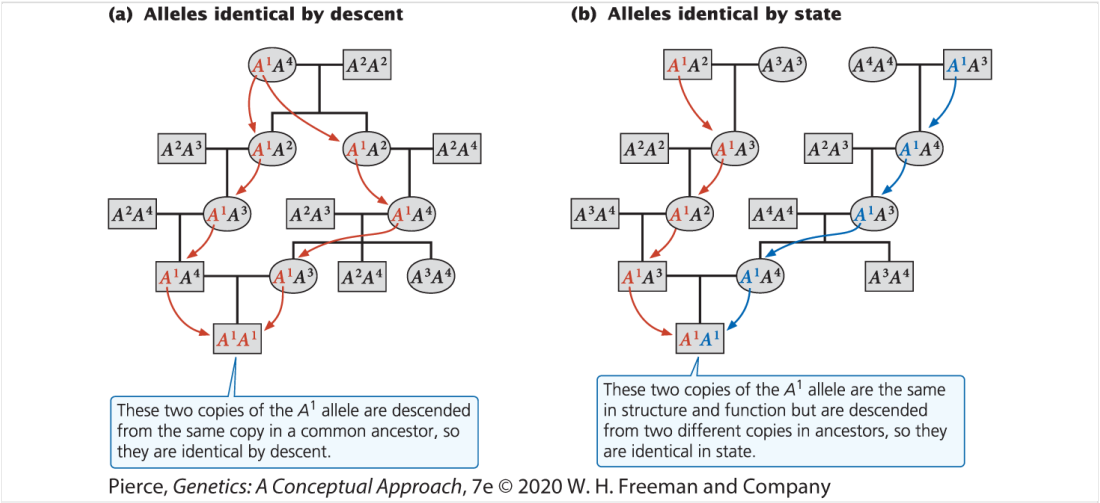
We can distinguish between two types of nonrandom mating. **Positive assortative mating** refers to a tendency for like individuals to mate. For example, humans exhibit positive assortative mating for height: tall people mate preferentially with other tall people; short people mate preferentially with other short people. **Negative assortative mating** refers to a tendency for unlike individuals to mate. If people engaged in negative assortative mating for height, tall and short people would preferentially mate. Assortative mating is usually for a particular trait and affects only those genes that encode the trait (and genes closely linked to them).

THINK-PAIR-SHARE Question 2

One form of nonrandom mating is **inbreeding**, which is preferential mating between related individuals. Inbreeding is actually positive assortative mating for relatedness, but it differs from other types of assortative mating because it affects all genes, not just those that determine the trait for which the mating preference exists. Inbreeding causes a departure from the Hardy–Weinberg equilibrium frequencies of p^2, $2pq$, and q^2. More specifically, it leads to an increase in the proportion of homozygotes and a decrease in the proportion of heterozygotes in a population. **Outcrossing** is preferential mating between unrelated individuals.

In a diploid organism, a homozygous individual has two copies of the same allele. These two copies may be the same in *state*, which means that the two alleles are alike in structure and function but do not have a common origin. Alternatively, the two

alleles in a homozygous individual may be the same because they are identical by *descent*; that is, the copies are descended from a single allele that was present in an ancestor (Figure 25.5). If we go back far enough in time, many alleles are likely to be identical by descent, but for calculating the effects of inbreeding, we consider identity by descent going back only a few generations.



25.5 Individuals may be homozygous by descent or by state. Inbreeding is a measure of the probability that two alleles are identical by descent.

Description

Pedigree chart a shows alleles identical by descent. The P generation shows a female with genotype big A superscript 1 (highlighted in red) big A superscript 4 mates with a male with genotype big A superscript 2 big A superscript 2. They have two daughters with the same genotype: big A superscript 1 (highlighted in red) big A superscript 2 in generation 1 below. The first daughter with genotype big A superscript 1 (highlighted in red) big A superscript 2 mates with a male with genotype big A superscript 2 big A superscript 3 and has a daughter with genotype big A superscript 1 (highlighted in red) big A superscript 3 in the generation 2 below. The other daughter in generation 1 with genotype big A superscript 1 (highlighted in red) big A superscript 2 mates with a male with genotype big A superscript 2 big A superscript 4 and has a daughter with genotype big A superscript 1 (highlighted in red) big A superscript 4 in generation 2 below. The daughter in generation 2 with genotype big A superscript 1 (highlighted in red) big A superscript 3 mates with a male with genotype big A superscript 2 big A superscript 4 and has a male with genotype big A superscript 1 (highlighted in red) big A superscript 4 in generation 3. The daughter with genotype big A superscript 1 (highlighted in red) big A superscript 4 in generation 2 mates with a male with genotype big A superscript 2 big A superscript 3 and has three offspring: a female with genotype big A superscript 1 (highlighted in red) big A superscript 3, a male with genotype big A superscript 2 big A superscript 4, and a female with genotype big A superscript 3 big A superscript 4 in generation 3. The male with genotype big A superscript 1 (highlighted in red) big A superscript 4 from generation 3 mates with his female cousin with genotype big A superscript 1 (highlighted in red) big A superscript 3 and they have a male offspring with genotype big A superscript 1 (highlighted in red) big A superscript 1 (highlighted in red) in generation 4 below.

A caption reads, These two copies of the big A superscript 1 allele are descended from the same copy in a common ancestor, so they are identical by descent. All the big A superscript 1 (highlighted in red) in the pedigree chart are connected by two arrows beginning from big A superscript 1 (highlighted in red) from generation P.

Pedigree chart b shows alleles identical by state.

The P generation at the top shows a male with genotype big A superscript 1 (highlighted in red) big A superscript 2 mates with a female with genotype big A superscript 3 big A superscript 3 and have a daughter with genotype big A superscript 1 (highlighted in red) big A superscript 3 in generation 1 below. Another family in P generation shows a female with genotype big A superscript 4 big A superscript 4 mates with a male with genotype big A superscript 1 (highlighted in blue) big A superscript 3 and have a daughter with genotype big A superscript 1 (highlighted in blue) big A superscript 4 in generation 1 below. The female with genotype big A superscript 1 (highlighted in red) big A superscript 3 from generation 1 mates with a male big A superscript 2 big A superscript 2 and has a daughter with genotype big A superscript 1 (highlighted in red) big A superscript 2 in generation 2 below. The female with genotype big A superscript 1 (highlighted in blue) big A superscript 4 from generation 1 mates with a male with genotype big A superscript 2 big A superscript 3 and has a daughter with genotype big A superscript 1 (highlighted in blue) big A superscript 3 in generation 2 below. The female with genotype big A superscript 1 (highlighted in red) big A superscript 2 from generation 2 mates with a male with genotype big A superscript 3 big A superscript 4 and have a son with genotype A superscript 1 (highlighted in red) big A superscript 3 in generation 3 below. The female with genotype big A superscript 1 (highlighted in blue) big A superscript 3 from generation 2 mates with a male big A superscript 4 big A superscript 4 and has a daughter with genotype big A superscript 1 (highlighted in blue) big A superscript 4 and a son with genotype big A superscript 3 big A superscript 4 in generation 3 below. The male with genotype big A superscript 1 (highlighted in red) big A superscript 3 from generation 3 mates with the female with genotype big A superscript 1 (highlighted in blue) big A superscript 4 from generation 3 and they have a son with genotype big A superscript 1 (highlighted in red) big A superscript 1 (highlighted in blue) in generation 4 below. A caption reads, These two copies of the big A superscript 1 allele are the same in structure and function, but are descended from two different copies in ancestors, so they are identical in state. All the big A superscript 1 (highlighted in red) in the pedigree chart are connected by an arrow beginning from big A superscript 1 (highlighted in red) from generation P. All the big A superscript 1 (highlighted in blue) in the pedigree chart are connected by an arrow beginning from big A superscript 1 (highlighted in blue) from generation P.

Inbreeding is usually measured by the **inbreeding coefficient**, designated *F*, which is a measure of the probability that two alleles are identical by descent. Inbreeding coefficients can range from 0 to 1. A value of 0 indicates that mating is occurring randomly in a large population; a value of 1 indicates that all alleles are identical by descent. Inbreeding coefficients can be calculated from analyses of pedigrees, or they can be determined from the reduction in the heterozygosity of a population. Although we will not go into the details of how *F* is calculated, an understanding of how inbreeding affects genotypic frequencies is important.

When inbreeding takes place, the proportion of heterozygotes decreases by $2Fpq$, and half of this value (Fpq) is *added* to the proportion of each homozygote each generation. The frequencies of the genotypes will then be as follows:

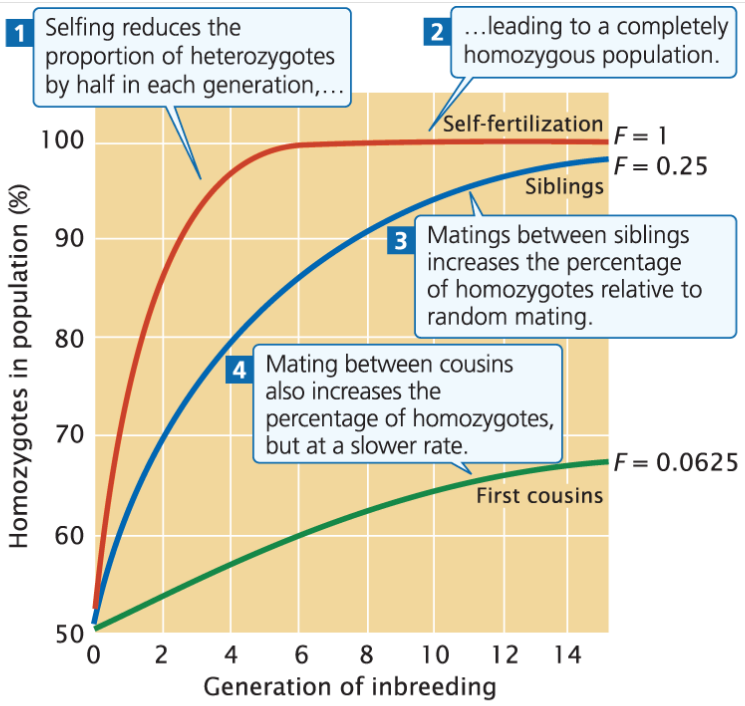
$$\begin{aligned} f(AA) &= p^2 + Fpq \\ f(Aa) &= 2pq - 2Fpq \\ f(aa) &= q^2 + Fpq \end{aligned} \tag{25.10}$$

Consider a population that reproduces by self-fertilization ($F = 1$). We will assume that this population begins with genotypic frequencies in Hardy–Weinberg proportions (p^2, $2pq$, and q^2). With selfing, each homozygote produces only progeny of the same homozygous genotype ($AA \times AA$ produces all AA; $aa \times aa$ produces all aa), whereas only half the progeny of a heterozygote has the same genotype as the parent ($Aa \times Aa$ produces $^1/_4\ AA$, $^1/_2\ Aa$, and $^1/_4\ aa$). Selfing therefore reduces the proportion of heterozygotes in the population by half with each generation, until all genotypes in the population are homozygous (Table 25.2 and Figure 25.6). Although all individuals in the population are homozygous at this point, both types of homozygotes (AA and aa) are still present, provided neither homozygote has an advantage over the other (there are no selective differences). › **TRY PROBLEM 29**

TABLE 25.2 Generational increase in frequency of homozygotes in a self-fertilizing population starting with $p = q = 0.5$

	Genotypic Frequencies		
Generation	***AA***	***Aa***	***aa***
1	$^1/_4$	$^1/_2$	$^1/_4$
2	$^1/_4 + ^1/_8 = ^3/_8$	$^1/_4$	$^1/_4 + ^1/_8 = ^3/_8$
3	$^3/_8 + ^1/_{16} = ^7/_{16}$	$^1/_8$	$^3/_8 + ^1/_{16} = ^7/_{16}$

4	$7/16 + 1/32 = 15/32$	$1/16$	$7/16 + 1/32 = 15/32$
n	$\frac{1-(1/2)^n}{2}$	$\left(1/2\right)^n$	$\frac{1-(1/2)^n}{2}$
∞	$1/2$	0	$1/2$



25.6 Inbreeding increases the percentage of homozygous individuals in a population.

Description

The horizontal axis represents generation of inbreeding and ranges from 0 to 14, in increments of 2. The vertical axis represents Homozygotes in population in percent and ranges from 50 to 100, in increments of 10. A line representing self fertilization F equals 1 starts from (0, 50), reaches (6, 100) and ends at (15, 100). A caption reads, Selfing reduces the proportion of heterozygotes by half in each generation leading to a completely homozygous population. A concave down increasing curve representing siblings F equals 0 point 2 5 begins at (0, 50) and ends at (15, 98). A caption reads, Matings between siblings increases the percentage of homozygotes relative to random mating. Another curve, slightly concave down increasing, representing first cousins F equals 0 point 0 6 2 5 starts from (0, 50) and ends at (15, 67). A caption reads, Mating between cousins also increases the percentage of homozygotes, but at a slower rate. All values are approximate.

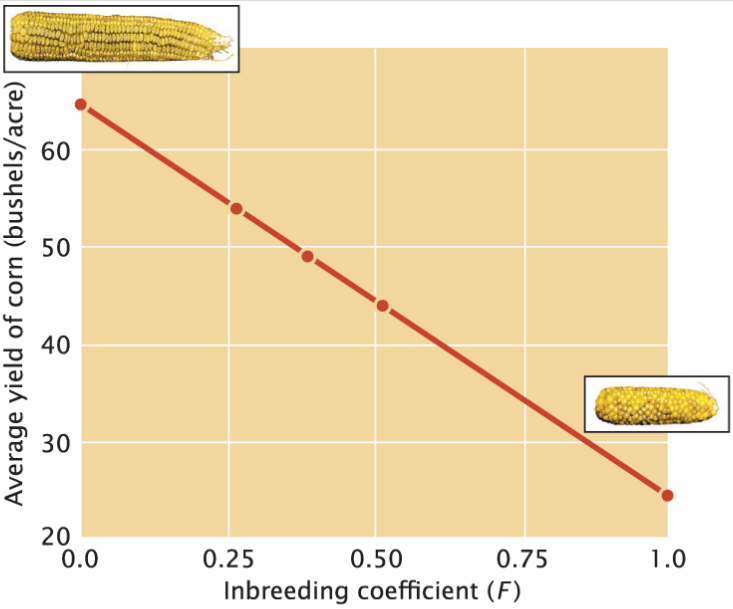
For most outcrossing species, close inbreeding is harmful because it increases the proportion of homozygotes and thereby boosts the probability that deleterious and lethal recessive alleles will combine to produce homozygotes with a harmful trait. Assume that a recessive allele (a) that causes a genetic disease has a frequency (q) of 0.01. If the population mates randomly ($F = 0$), the frequency of individuals affected by the disease (aa) will be $q^2 = 0.01^2 = 0.0001$, so only 1 in 10,000 individuals will have the disease. However, if $F = 0.25$ (the equivalent of brother–sister mating), then the expected frequency of the aa homozygote is $q^2 + Fpq = (0.01)^2 + (0.25)(0.99)(0.01) = 0.0026$; thus, the genetic disease is 26 times as frequent at this level of inbreeding. This increased appearance of lethal and deleterious traits with inbreeding is termed **inbreeding depression**; the more intense the inbreeding, the more severe the inbreeding depression.

The harmful effects of inbreeding have been recognized for thousands of years and may be the basis of cultural taboos against mating between close relatives. William Schull and James Neel found that for each 10% increase in F, the mean IQ of Japanese children dropped six points. Child mortality also increases with close inbreeding (Table 25.3); children of first cousins have a 40% increase in mortality over that seen among the children of unrelated people. Similarly, studies conducted on over 11,000 babies born between 2007 and 2011 in Bradford, Great Britain, found that the risk of congenital anomalies was twice as high among children whose parents were first cousins than among those whose parents were unrelated. Inbreeding also has deleterious effects on crops (Figure 25.7) and domestic animals.

TABLE 25.3 Effects of inbreeding on Japanese children

Genetic Relationship of Parents	*F*	Mortality of Children (through 12 years of age)
Unrelated	0	0.082
Second cousins	0.016 ($^1/_{64}$)	0.108
First cousins	0.0625 ($^1/_{16}$)	0.114

Data from D. L. Hartl and A. G. Clark, *Principles of Population Genetics*, 2d ed. (Sunderland, Mass.: Sinauer Associates, 1989), Table 2. Original data from W. J. Schull and J. V. Neel, *The Effects of Inbreeding on Japanese Children* (New York: Harper & Row, 1965).



25.7 Inbreeding often has deleterious effects on crops. As inbreeding increases, the average yield of corn decreases.

Description

The horizontal axis represents inbreeding coefficient (F) and ranges from 0 point 0 to 1 point 0, in increments of 0 point 2 5. The vertical axis represents average yield of corn (bushels per acre) and ranges from 20 to 60, in increments of 10. A line with negative slope begins at (0 point 0, 60) and reaches (1 point 0, 25). Five points plotted on the line are as flows: (0 point 0, 65), (0 point 26, 54), (0 point 3 7 5, 49 point 5), (0 point 5 1, 44), and (1 point 0, 24). A photo shows a longer corn cob at the beginning of the line and another photo shows a short corn cob at the end of the line. All values are approximate.

Inbreeding depression is most often studied in humans, as well as in plants and animals reared in captivity, but the negative effects of inbreeding may be more severe in natural populations. Julie Jimenez and her colleagues collected wild mice from a natural population in Illinois and bred them in the laboratory for three to four generations. Laboratory matings were chosen so that some mice had no inbreeding, whereas others had an inbreeding coefficient of 0.25. When both types of mice were released back into the wild, the weekly survival rate of the inbred mice was only 56%

of that of the non-inbred mice. Inbred male mice also continuously lost body weight after release into the wild, whereas non-inbred male mice initially lost weight, but then regained it within a few days after release.

In spite of the fact that inbreeding is generally harmful for outcrossing species, a number of plants and animals regularly inbreed and are successful (**Figure 25.8**). As stated earlier, inbreeding increases homozygosity, and eventually all individuals in the population become homozygous. If a species undergoes inbreeding for a number of generations, many deleterious recessive alleles are weeded out by natural or artificial selection, so the population becomes homozygous for beneficial alleles. In this way, the harmful effects of inbreeding may eventually be eliminated, leaving a population that is homozygous for beneficial traits. In addition, inbreeding helps to preserve groups of genes (called co-adapted gene complexes) that exhibit gene interaction and work well together in a specific environment. Outcrossing, on the other hand, breaks up these co-adapted gene complexes and leads to the recombination of genes that might not work well together. In this way, inbreeding may be favored.

THINK-PAIR-SHARE Question 3

Huetter, C/Age Fotostock America, Inc.

25.8 Although inbreeding is generally harmful, a number of inbreeding organisms are successful. Shown here is the terrestrial slug *Arion circumscriptos*, an inbreeding species that causes damage in greenhouses and flower gardens.

CONCEPTS

Nonrandom mating alters the frequencies of genotypes but not the frequencies of alleles. Inbreeding is preferential mating between related individuals. With inbreeding, the frequency of homozygotes increases, whereas the frequency of heterozygotes decreases.

✔ CONCEPT CHECK 5

What is the effect of outcrossing on a population?

a. Allelic frequencies change.
b. There will be more heterozygotes than predicted by the Hardy–Weinberg law.
c. There will be fewer heterozygotes than predicted by the Hardy–Weinberg law.
d. Genotypic frequencies will equal those predicted by the Hardy–Weinberg law.

25.4 Several Evolutionary Forces Can Change Allelic Frequencies

The Hardy–Weinberg law indicates that allelic frequencies do not change as a result of reproduction. The processes that bring about change in allelic frequencies include mutation, migration, genetic drift (random effects due to small population size), and natural selection.

Mutation

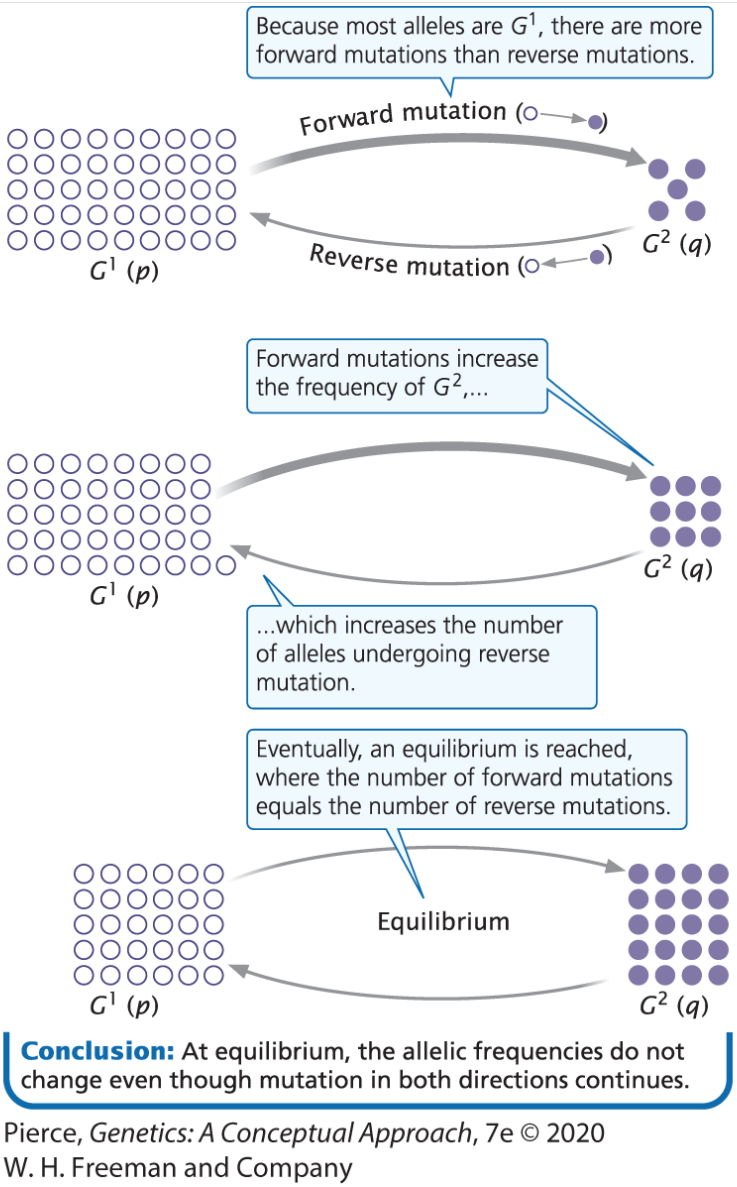
Before evolution can take place, genetic variation must exist within a population; consequently, all evolution depends on processes that generate genetic variation. Although new *combinations* of existing genes may arise through recombination in meiosis, all genetic variants ultimately arise through mutation.

THE EFFECT OF MUTATION ON ALLELIC FREQUENCIES

Mutation can influence the rate at which one genetic variant increases at the expense of another. Consider a single locus in a population of 25 diploid individuals. Each individual possesses two alleles at the locus under consideration, so the gene pool of the population consists of 50 allele copies. Let's assume that there are two different alleles, designated G^1 and G^2, with frequencies p and q, respectively. If there are 45 copies of G^1 and 5 copies of G^2 in the population, $p = 0.90$ and $q = 0.10$. Now suppose that a mutation changes a G^1 allele into a G^2 allele. After this mutation, there are 44 copies of G^1 and 6 copies of G^2, and the frequency of G^2 has increased from 0.10 to 0.12. Mutation has changed the allelic frequencies.

If copies of G^1 continue to mutate to G^2, the frequency of G^2 will increase and the frequency of G^1 will decrease (Figure 25.9). The amount by which G^2 will change (Δq) as a result of mutation depends on (1) the rate of G^1-to-G^2 mutation (μ) and (2) p, the frequency of G^1 in the population. When p is large, there are many copies of G^1 available to mutate to G^2, and the amount of change will be relatively large. As more mutations occur and p decreases, there will be fewer copies of G^1 available to mutate to G^2. The change in G^2 as a result of mutation equals the mutation rate times the allelic frequency:

$$\Delta q = \mu p \tag{25.11}$$



25.9 Recurrent mutation changes allelic frequencies. Forward and reverse mutations eventually lead to equilibrium.

Description

The illustration shows an array of 45 blue outlined circular structures in 5 rows and 9 columns, named big G superscript 1 open parenthesis p close parenthesis on the top left side. A forward arrow represents forward mutation where blue outlined circular structures become blue filled circular structures. A caption reads, Because most alleles are big G superscript 1, there are more forward mutations than reverse mutations. The right side shows 5 blue filled circular structures named big G superscript 2 open parenthesis q close parenthesis. A backward arrow from blue filled structures to blue outline structures represents reverse mutation where blue filled structure changes to blue outlined structure.

An array of 41 outlined circular structures arranged in 5 rows, 8 columns and one sits at the right bottom end is labeled big G superscript 1 open parenthesis p close parenthesis. A forward arrow represents forward mutation. A caption reads, Forward mutations increase the frequency of big G superscript 1. An array of 9 blue filled circular structures is present on the right, arranged in 3 rows and 3 columns is labeled big G superscript 2 open parenthesis q close parenthesis. A backward arrow from blue filled structures to blue outline structures is present. A caption reads, which increases the number of alleles undergoing reverse mutation.

An array of 30 blue outlined circular structures arranged in 5 rows, 6 columns is labeled big G superscript 1 open parenthesis p close parenthesis. A forward arrow points to the right. An array of 20 blue filled circular structures arranged in 5 rows and 4 columns, labeled big G superscript 2 open parenthesis q close parenthesis is present on the right side. A backward arrow from blue filled structures to blue outline structures is present. Text between the arrows reads, equilibrium. A caption reads, eventually, an equilibrium is reached, where the number of forward mutations equals the number of reverse mutations.

A caption below reads, Conclusion: At equilibrium, the allelic frequencies do not change even though mutation in both directions continues.

As the frequency of p decreases as a result of mutation, the change in frequency due to mutation will be less and less.

So far, we have considered only the effects of $G^1 \rightarrow G^2$ forward mutations. Reverse $G^2 \rightarrow G^1$ mutations also occur at rate ν, which will probably be different from the forward mutation rate, μ. Whenever a reverse mutation occurs, the frequency of G^2 decreases and the frequency of G^1 increases (see Figure 25.9). The rate of change due to reverse mutations equals the reverse mutation rate times the allelic frequency of $G^2 (\Delta q = \nu q)$. The overall change in allelic frequencies is a balance between the opposing forces of forward mutation and reverse mutation:

$$\Delta q = \mu p - \nu q \tag{25.12}$$

REACHING EQUILIBRIUM OF ALLELIC FREQUENCIES

Consider a population that begins with a high frequency of G^1 and a low frequency of G^2. In this population, many copies of G^1 are initially available to mutate to G^2,

and the increase in G^2 due to forward mutation will be relatively large. However, as the frequency of G^2 increases as a result of forward mutations, fewer copies of G^1 are available to mutate, so the number of forward mutations decreases. On the other hand, few copies of G^2 are initially available to undergo reverse mutation to G^1, but as the frequency of G^2 increases, the number of copies of G^2 available to undergo reverse mutation to G^1 increases, so the number of genes undergoing reverse mutation will increase. Eventually, the number of genes undergoing forward mutation will be counterbalanced by the number of genes undergoing reverse mutation. At this point, the increase in q due to forward mutation will be equal to the decrease in q due to reverse mutation, and there will be no net change in allelic frequency ($\Delta q = 0$), in spite of the fact that forward and reverse mutations continue to occur. A point at which there is no change in the allelic frequencies of a population is referred to as an **equilibrium** (see Figure 25.9). At mutational equilibrium, the frequency of G^2 ($\hat{q}$) will be

$$(\hat{q}) = \frac{\mu}{\mu + v} \tag{25.13}$$

This final equation tells us that the allelic frequency at equilibrium is determined solely by the forward (μ) and reverse (v) mutation rates. › **TRY PROBLEM 31**

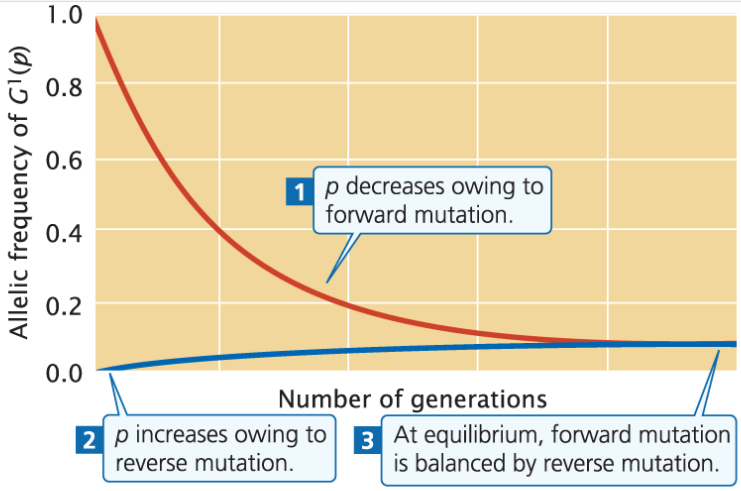
SUMMARY OF EFFECTS OF MUTATION

When the only evolutionary force acting on a population is mutation, allelic frequencies change over time because some alleles mutate into others. Eventually, these allelic frequencies reach equilibrium and are determined only by the forward and reverse mutation rates. The Hardy–Weinberg law tells us that when the allelic frequencies reach equilibrium, the genotypic frequencies will also remain the same.

The mutation rates for most genes are low, so change in allelic frequencies due to mutation in one generation is very small, and long periods are required for a population to reach mutational equilibrium. For example, if the forward and reverse mutation rates for alleles at a locus are 1×10^{-5} and 0.3×10^{-5} per generation, respectively (rates that have actually been measured at several loci in mice), and the allelic frequencies are $p = 0.9$ and $q = 0.1$, then the net change in allelic frequency per generation due to mutation is

$$\begin{aligned}\Delta q &= \mu p - v q \\ &= (1 \times 10^{-5})(0.9) - (0.3 \times 10^{-5})(0.1) \\ &= 8.7 \times 10^{-6} \\ &= 0.0000087\end{aligned}$$

Therefore, change due to mutation in a single generation is extremely small, and as the frequency of p drops as a result of mutation, the amount of change will become even smaller (Figure 25.10). The effect of typical mutation rates on Hardy–Weinberg equilibrium is negligible, and many generations are required for a population to reach mutational equilibrium. Nevertheless, if mutation is the only force acting on a population for long periods, mutation rates will determine allelic frequencies.



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

25.10 Change due to recurrent mutation slows as the frequency of *p* drops. Allelic frequencies approach mutational equilibrium at typical low mutation rates. The allelic frequency of G^1 decreases as a result of forward ($G^1 \rightarrow G^2$) mutation at a rate of 0.0001 and increases as a result of reverse ($G^2 \rightarrow G^1$) mutation at a rate of 0.00001. Owing to the low rate of mutations, eventual equilibrium takes many generations to be reached.

Description
The horizontal axis represents number of generations and the vertical axis represents allelic frequency of big G superscript 1 open parenthesis p close parenthesis. The vertical axis ranges from 0 point 0 to 1 point 0, in increments of 0 point 2. A concave up decreasing curve begins from 1 point 0 corresponding to the vertical axis and reaches almost the bottom right end of the graph. A caption reads, p decreases owing to forward mutation. A slightly concave down increasing curve begins over the origin, increases slightly and remains constant till it reaches the bottom right end of the graph where it overlaps the previous line. A caption reads, p increases owing to reverse mutation. At equilibrium, forward mutation is balanced by reverse mutation. All values are approximate.

CONCEPTS

Recurrent mutation causes changes in the frequencies of alleles. At equilibrium, allelic frequencies are determined by forward and reverse mutation rates. Because mutation rates are low, the effect of mutation on allelic frequencies per generation is very small.

✔ CONCEPT CHECK 6

When a population is in mutational equilibrium, which of the following is true?

a. The number of forward mutations is greater than the number of reverse mutations.
b. No forward or reverse mutations occur.
c. The number of forward mutations is equal to the number of reverse mutations.
d. The population is in Hardy–Weinberg equilibrium.

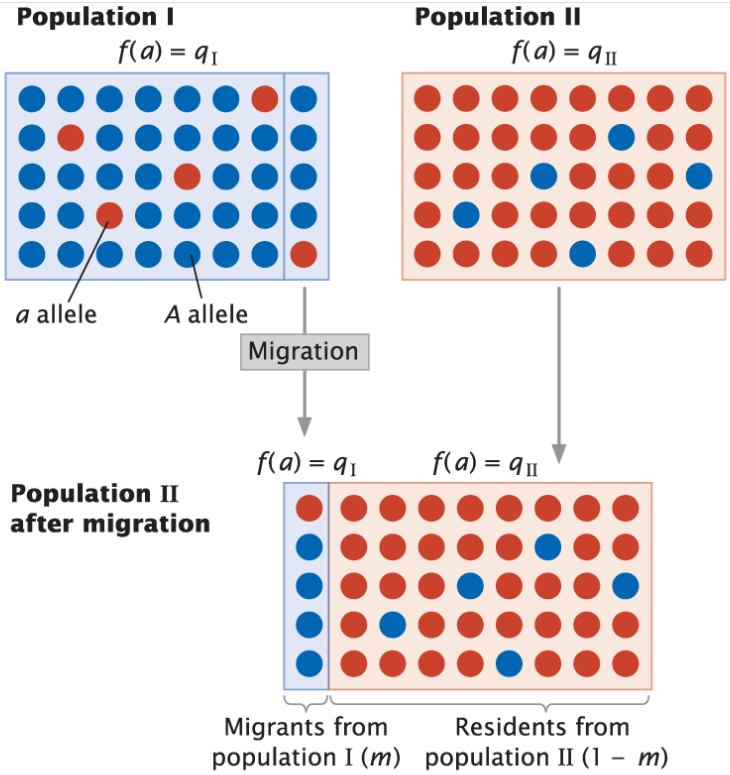
Migration

Another process that may bring about change in a population's allelic frequencies is the influx of genes from other populations, commonly called **migration** or **gene**

flow. One of the assumptions of the Hardy–Weinberg law is that migration does not take place, but many natural populations do experience migration from other populations. The overall effect of migration is twofold: (1) it prevents populations from becoming genetically different from one another, and (2) it increases genetic variation within populations.

THE EFFECT OF MIGRATION ON ALLELIC FREQUENCIES

Let's consider the effects of migration by looking at a simple, unidirectional model of migration between two populations that differ in the frequency of an allele *a*. Suppose that the frequency of this allele in population I is q_{I} and in population II is q_{II} (**Figure 25.11**). In each generation, a representative sample of the individuals in population I migrates to population II and reproduces, adding its alleles to population II's gene pool. Migration is only from population I to population II (unidirectional), and all the assumptions of the Hardy–Weinberg law (large population size, random mating, etc.) apply except the absence of migration.



25.11 The amount of change in allelic frequencies due to migration between populations depends on the difference between the populations in their allelic frequencies and on the extent of migration. Shown here is a model of the effect of unidirectional migration on allelic frequencies. The frequency of allele *a* in the source population (population I) is q_I. The frequency of this allele in the recipient population (population II) is q_{II}.

Description

The illustration shows population 1 at the top left. An array of 40 filled circular structures is arranged in 5 rows and 8 columns. Five red filled circular structures represent small A allele and are in the following positions: Row 2 column 2, Row 4 column 3, Row 3 column 5, Row 1 column 7, and Row 5 column 8. All the 35 remaining blue filled circular structures represent big A allele. Text at the top reads, f of open parenthesis a close parenthesis equals q subscript 1.

Population 2 at the top right shows an array of 40 filled circular structures, arranged in 5 rows and 8 columns. Five blue filled circular structures represent big A allele and are in the following positions: Row 4 column 2, Row 3 column 4, Row 5 column 5, Row 2 column 6, and Row 3 column 8. All the 35 remaining red filled circular structures represent small A allele. Migration occurs below. Text at the top reads, f of open parenthesis a close parenthesis equals q subscript 2.

Population 2 after migration below shows an array of 45 filled circular structures, arranged in 5 rows and 9 columns. The first column for all the rows is labeled Migrants from population 1 open parenthesis m close parenthesis, f of open parenthesis a close parenthesis equals q subscript 1. Row 1 column 1 shows a red filled circular structure and all the remaining rows for column 1 have a blue filled circular structure. The second column to ninth column has same entries for all the rows that were in the population 2. Column 2 to column 9 for all the rows are labeled Residents from population 2 open parenthesis 1 minus m close parenthesis, f of open parenthesis a close parenthesis equals q subscript 2.

A caption below reads, Conclusion: The frequency of allele a in population 2 after migration is q prime 2 equals q subscript 1 m plus q subscript 2 open parenthesis 1 minus m close parenthesis.

After migration, population II consists of two types of individuals. Some are migrants; they make up proportion m of population II, and they carry alleles from population I, so the frequency of allele a in the migrants is q_{I}. The other individuals in population II are the original residents. If the migrants make up proportion m of population II, then the residents make up proportion 1 – m; because the residents originated in population II, the frequency of allele a in this group is q_{II}. After migration, the frequency of allele a in the merged population II (q'_{II}) is

$$q'_{\mathrm{II}} = q_{\mathrm{I}}(m) + q_{\mathrm{II}}(1-m)$$

(25.14)

where $q_{\mathrm{I}}(m)$ is the contribution to q made by the copies of allele a in the migrants and $q_{\mathrm{II}}(1 - m)$ is the contribution to q made by the copies of allele a in the residents. The change in the allelic frequency due to migration (Δq) will be

$$\Delta q = m(q_{\mathrm{I}} - q_{\mathrm{II}})$$

(25.15)

Equation 25.15 summarizes the factors that determine the amount of change in allelic frequency due to migration. The amount of change in q is directly proportional to the amount of migration (m); as the amount of migration increases, the change in allelic frequency increases. The magnitude of change is

also affected by the difference in allelic frequencies between the two populations ($q_I - q_{II}$); when the difference is large, the change in allelic frequency will be large.

With each generation of migration, the allelic frequencies of the two populations become more and more similar until, eventually, the frequency of population II equals that of population I. When $q_I - q_{II} = 0$, there will be no further change in the allelic frequency of population II, in spite of the fact that migration continues. If migration between two populations takes place for a number of generations with no other evolutionary forces present, an equilibrium is reached at which the allelic frequencies of the recipient population equal those of the source population.

This simple model of unidirectional migration between two populations can be expanded to accommodate multidirectional migration between several populations.

THINK-PAIR-SHARE Question 4

THE OVERALL EFFECT OF MIGRATION

Migration has two major effects. First, it causes the gene pools of different populations to become more similar. Later in this section, we will see how genetic drift and natural selection lead to genetic differences between populations; migration counteracts these evolutionary forces and tends to keep populations homogeneous in their allelic frequencies. Second, migration adds genetic variation to populations. Different alleles may arise in different populations owing to rare mutational events, and these alleles can be spread to new populations by migration, increasing the genetic variation within the recipient population. **› TRY** PROBLEM 35

THINK-PAIR-SHARE Question 5

CONCEPTS

Migration causes changes in the allelic frequencies of a population by introducing alleles from other populations. The amount of change due to migration depends on both the amount of migration and the difference in allelic frequencies between the source and the recipient populations. Migration decreases genetic differences between populations and increases genetic variation within populations.

✔ CONCEPT CHECK 7

In each generation, 10 random individuals migrate from population A to population B. What will happen to allelic frequency q as a result of migration when q is equal in populations A and B?

a. q in A will decrease.
b. q in B will increase.
c. q will not change in either A or B.
d. q in B will become q^2.

Genetic Drift

The Hardy–Weinberg law assumes random mating in an infinitely large population; only when population size is infinite will the gametes carry genes that perfectly represent the parental gene pool. But no real population is infinitely large, and when population size is limited, the gametes that unite to form individuals of the next generation carry a sample of the alleles present in the parental gene pool. Just by chance, the composition of this sample often deviates from that of the parental gene pool, and this deviation may cause allelic frequencies to change. The smaller the gametic sample, the greater the chance that its composition will deviate from that of the parental gene pool.

The role of chance in altering allelic frequencies is analogous to the flip of a coin. Each time we flip a coin, we have a 50% chance of getting a head and a 50% chance of getting a tail. If we flip a coin 1000 times, the observed ratio of heads to

tails will be very close to the expected 50:50 ratio. If, however, we flip a coin only 10 times, there is a good chance that we will obtain not exactly five heads and five tails but maybe seven heads and three tails, or eight tails and two heads. This kind of deviation from an expected ratio due to limited sample size is referred to as **sampling error**.

Sampling error arises when gametes unite to produce progeny. Many organisms produce a large number of gametes, but when population size is small, a limited number of gametes unite to produce the individuals of the next generation, and chance influences which alleles are present in this limited sample. In this way, sampling error may lead to **genetic drift**, or changes in allelic frequencies. Because the deviations from the expected ratios are random, the direction of change is unpredictable. We can nevertheless predict the magnitude of the changes.

THE MAGNITUDE OF GENETIC DRIFT

The effects of genetic drift over time can be viewed in two ways. First, we can see how genetic drift changes the allelic frequencies of a single population. Second, we can see how it affects differences that accumulate among a series of populations. Imagine that we have 10 small populations, all beginning with the same allelic frequencies of $p = 0.5$ and $q = 0.5$. When genetic drift occurs in a population, allelic frequencies within the population will change, but because genetic drift is random, the ways in which allelic frequencies change in each population will not be the same. In some populations, p may increase as a result of chance. In other populations, p may decrease as a result of chance. In time, the allelic frequencies in the 10 populations will become different: the populations will diverge genetically over time. The change in allelic frequencies within each population and the genetic divergence among populations are due to the same force: the random change in allelic frequencies. Thus, the magnitude of genetic drift can be assessed either by examining the change in allelic frequencies within

a single population or by examining the magnitude of genetic differences that accumulate among populations.

The amount of genetic drift can be estimated from the variance in allelic frequency. Variance (s^2) is a statistical measure that describes the variability of a trait (see p. 739 in Chapter 24). Suppose that we observe a large number of separate populations, each with N individuals and allelic frequencies of p and q. After one generation of random mating, genetic drift—expressed in terms of the variance in allelic frequency among the populations (s_p^2)—will be

$$s_p^2 = \frac{pq}{2N} \tag{25.16}$$

The amount of change resulting from genetic drift (the variance in allelic frequency) is determined by two parameters: the allelic frequencies (p and q) and the population size (N). Genetic drift is maximal when p and q are equal (each 0.5). For example, assume that a population consists of 50 individuals. When the allelic frequencies are equal ($p = q = 0.5$), the variance in allelic frequency (s_p^2) will be $(0.5 \times 0.5)/(2 \times 50) = 0.0025$. In contrast, when $p = 0.9$ and $q = 0.1$, the variance in allelic frequency will be only 0.0009. Genetic drift will also be greater when the population size is small. If $p = q = 0.5$, but the population size is only 10, then the variance in allelic frequency becomes $(0.5 \times 0.5)/(2 \times 10) = 0.0125$, which is five times greater than when the population size is 50.

This divergence of populations through genetic drift is strikingly illustrated in the results of an experiment carried out by Peter Buri on fruit flies (**Figure 25.12**). Buri examined the frequencies of two alleles (bw^{75} and bw) that affect eye color in fruit flies. He set up 107 replicate populations, each consisting of eight males and eight females. He began each population with a frequency of bw^{75} equal to 0.5. He allowed the flies within each replicate population to mate randomly, and from each generation, he randomly selected eight male and eight female flies to be the

parents of the next generation. He followed the changes in the frequencies of the two alleles over 19 generations. In one population, for example, the average frequency of bw^{75} (p) over the 19 generations was 0.53125. We can use Equation 25.16 to calculate the expected variance in allelic frequency due to genetic drift. The frequency of the *bw* allele (q) is 1 – p = 1 – 0.53125 = 0.46875. The population size (N) equals 16. The expected variance in allelic frequency is therefore

$$\frac{pq}{2N} = \frac{0.53125 \times 0.46875}{2 \times 16} = 0.0156$$

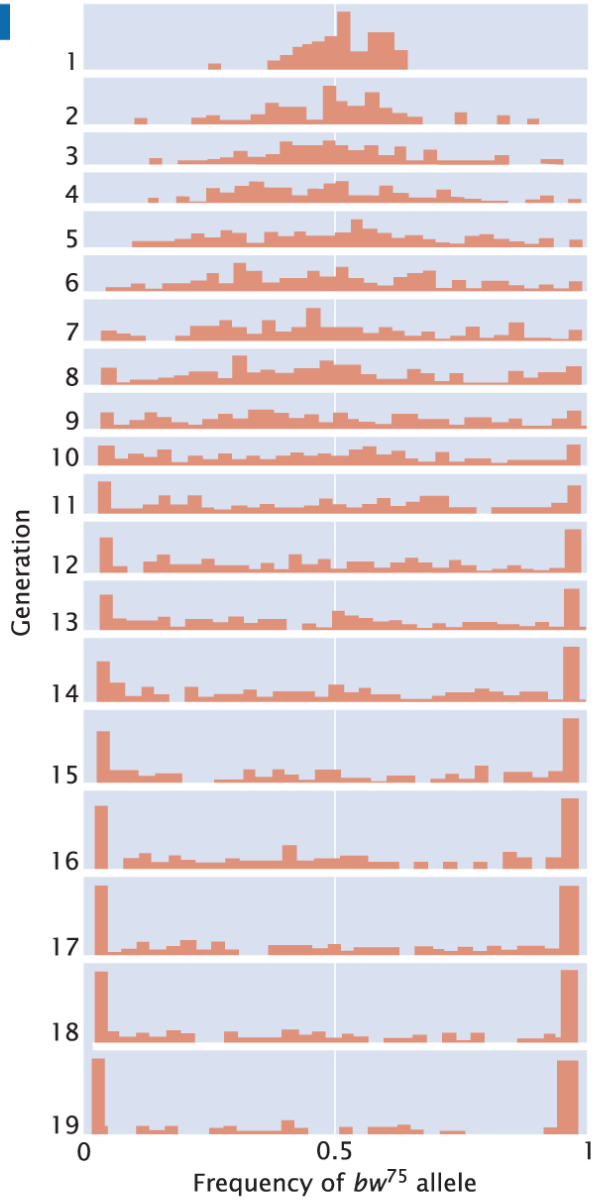
which was very close to the actual observed variance of 0.0151.

Experiment

Question: What effect does genetic drift have on the genetic composition of populations?

Methods Buri examined the frequencies of two alleles (bw^{75} and *bw*) that affect *Drosophila* eye color in 107 small replicate populations over 19 generations.

Results



Conclusion: As a result of genetic drift, allelic frequencies in the different populations diverged and often became fixed for one allele or the other.

Pierce, *Genetics: A Conceptual Approach*, 7e © 2020
W. H. Freeman and Company

25.12 Populations diverge in allelic frequency and become fixed for one allele as a result of genetic drift. In Buri's study of two eye-color alleles (bw^{75} and *bw*) in *Drosophila*, each population consisted of eight males and eight females and began with the frequency of bw^{75} equal to 0.5.

Description

The illustration shows a question that reads, What effect does genetic drift have on the genetic composition of populations? The methods section below reads, Buri examined the frequencies of two alleles (small B small W superscript 75 and small B small W) that affect Drosophila eye color in 107 replicate small populations over 19 generations. The results section below shows 19 bar graphs labeled 1 to 19, each representing its corresponding generation. The vertical axes of all the graphs represent generation. The horizontal axes of all the graphs represent frequency of small B small W superscript 75 gene and ranges from 0 to 1, in increments of 0 point 5.

Graph 1 shows most of its large bars over the center of the horizontal axis at 0 point 5.

Graph 2 below shows most of its large bars over the center of the horizontal axis at 0 point 5 and few spreading more towards the end of the graph.

Graph 3 below shows small bars over the center of the horizontal axis at 0 point 5 and few spreading more towards the end of the graph.

Graph 4 below shows bars, which are small over the center of the horizontal axis at 0 point 5 and few spreading more towards the right end of the graph.

Graph 5 below shows small bars spreading all over the graph.

Graph 6 below shows small bars spreading all over the graph.

Graph 7 below shows small bars spreading all over the graph. Few bars around the center of the horizontal axis are longer.

Graph 8 below shows small bars spreading all over the graph. One longer bar is at each end of the graph.

Graph 9 below shows minute bars spreading all over the graph. One bar is at each end of the graph.

Graph 10 below shows minute bars spreading all over the graph. One longer bar is at each end of the graph.

Graph 11 below shows minute bars spreading all over the graph. One longer bar is at each end of the graph while the bars at the center are relatively small.

Graph 12 below shows minute bars spreading all over the graph. One longer bar is at each end of the graph while the bars at the center are bit small.

Graph 13 below shows minute bars spreading all over the graph. One longer bar is at each end of the graph while the bars at the center are bit small.

Graph 14 below shows minute bars spreading all over the graph. One very long bar is at each end of the graph while the bars at the center are considerably small.

Graph 15 below shows minute bars spreading all over the graph. One very long bar is at each end of the graph. There are few random spaces between the bars.

Graph 16 below shows minute bars spreading all over the graph. One very long bar is at each end of the graph. There are few random spaces between the bars.

Graph 17 below shows minute bars spreading all over the graph. One very long bar is at each end of the graph. There are few random spaces between the bars.

Graph 18 below shows minute bars spreading all over the graph. One very long bar is at each end of the graph. There are few random spaces between the bars.

Graph 19 below shows minute bars spreading all over the graph. One very long bar is at each end of the graph. There are few random large spaces between the bars.

A caption below reads, Conclusion: As a result of genetic drift, allelic frequencies in the different populations diverged and often became fixed for one allele or the other.

The effect of population size on genetic drift is illustrated by a study conducted by Luca Cavalli-Sforza and his colleagues. They studied variation in blood types among villagers in the Parma Valley of Italy, where migration between villages was limited. They found that variation in allelic frequency was greatest between small, isolated villages in the upper valley, and less so between larger villages and towns farther down the valley. This result is exactly what we expect with genetic drift: there should be more genetic drift, and thus more variation, among populations when population sizes are small.

For ecological and demographic studies, population size is usually defined as the number of individuals in a group. However, the evolution of a gene pool depends only on those individuals who contribute genes to the next generation. Population geneticists usually define population size as the equivalent number of breeding adults, referred to as the **effective population size** (N_e). Several factors determine the equivalent number of breeding adults, including the sex ratio, the variation between individuals in number of offspring produced, fluctuations in population size, the age structure of the population, and whether mating is random.

CONCEPTS

Genetic drift is changes in allelic frequencies due to chance. The amount of change in allelic frequencies due to genetic drift is inversely related to the effective population size (the equivalent number of breeding adults in a population).

✔ CONCEPT CHECK 8

Which of the following statements describes an example of genetic drift?

a. Allele *g* for fat production increases in a small population because birds with more body fat have higher survivorship in a harsh winter.
b. Random mutation increases the frequency of allele *A* in one population but not in another.
c. Allele *R* reaches a frequency of 1.0 because individuals with genotype *rr* are sterile.
d. Allele *m* is lost when a virus kills all but a few individuals, and just by chance, none of the survivors possess allele *m*.

CAUSES OF GENETIC DRIFT

All genetic drift arises from sampling error, but there are several different ways that sampling error can arise. First, a population may be reduced in size for a number of generations because of limitations in space, food, or some other critical resource. Genetic drift in a small population over multiple generations can significantly affect the composition of a population's gene pool.

A second way that sampling error can arise is through the **founder effect**, which results from the establishment of a population by a small number of individuals; the population of wolves on Isle Royale, discussed in the introduction to this chapter, underwent a founder effect. Although the population may increase and become quite large, the genes carried by all of its members are derived from the few genes originally present in the founders (assuming no migration or mutation). Chance events affecting which genes were present in the founders have an important influence on the makeup of the entire population.

A third way in which genetic drift arises is through a **genetic bottleneck**, which develops when a population undergoes a drastic reduction in size. An example is seen in northern elephant seals (**Figure 25.13**). Before 1800, thousands of northern elephant seals were found along the California coast, but hunting between 1820 and 1880 devastated the population. By 1884, as few as 20 seals survived on a remote beach on Isla de Guadalupe, west of Baja California, Mexico. Restrictions on hunting enacted by the United States and Mexico allowed the seals to recover, and there are now more than 100,000 seals in the population. All the seals in the population today are genetically similar, however, because they have only those genes that were carried by the few survivors of the population bottleneck.

THINK-PAIR-SHARE Question 6

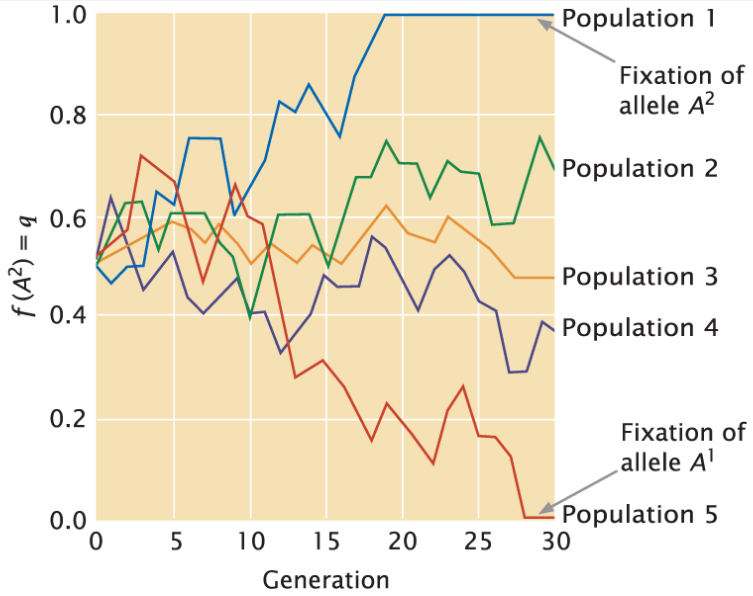
National Geographic Image Collection/Alamy Stock Photo.

25.13 Northern elephant seals underwent a severe genetic bottleneck between 1820 and 1880.
Today, these seals have low levels of genetic variation.

THE EFFECTS OF GENETIC DRIFT

Genetic drift has several important effects on the genetic composition of a population. First, it produces change in allelic frequencies within a population.

Because genetic drift is random, the frequency of any allele is just as likely to increase as it is to decrease and will wander with the passage of time (hence the name *genetic drift*). **Figure 25.14** illustrates a computer simulation of genetic drift in five populations over 30 generations, starting with $q = 0.5$ and maintaining a constant population size of 10 males and 10 females. The allelic frequencies in these populations change randomly from generation to generation.



25.14 Genetic drift changes allelic frequencies within populations, leading to a reduction in genetic variation through fixation and genetic divergence among populations. Shown here is a computer simulation of changes in the frequency of allele $A^2(q)$ in five different populations due to random genetic drift. Each population consists of 10 males and 10 females and begins with $q = 0.5$.

Description

The horizontal axis represents generation and ranges from 0 to 30, in increments of 5. The vertical axis represents f of open parenthesis big A superscript 2 close parenthesis equals q and ranges from 0 point 0 to 1 point 0, in increments of 0 point 2. A line representing population 1 begins at (0, 0 point 5), reaches (18, 1 point 0) after larger number of fluctuations, and ends at (30, 1 point 0). A caption reads, Fixation of allele big A superscript 2. A line representing population 2 starts from (0, 0 point 5), reaches (19, 0 point 7 5) after larger number of fluctuations, and ends at (30, 0 point 7). A line representing population 3 starts from (0, 0 point 5), reaches (19, 0 point 6 2), and ends at (30, 0 point 4 7) after larger number of fluctuations. A line representing population 4 starts from (0, 0 point 5), reaches (13, 0 point 3 2), (17, 0 point 5 6) and ends at (30, 0 point 3 9) after larger number of fluctuations. A line representing population 5 starts from (0, 0 point 5), reaches (9, 0

point 6 6), (24, 0 point 2 6) and ends at (30, 0 point 0) after larger number of fluctuations. A caption reads, Fixation of allele big A superscript 1. All values are approximate.

A second effect of genetic drift is the reduction of genetic variation within populations. Through random change, an allele may eventually reach a frequency of either 1 or 0, at which point all individuals in the population are homozygous for one allele. When an allele has reached a frequency of 1, we say that it has reached **fixation**. Other alleles are lost (reach a frequency of 0) and can be restored only by migration from another population or by mutation. Fixation, then, leads to a loss of genetic variation within a population. Such losses can be seen in the northern elephant seals just described. Today, these seals have low levels of genetic variation; a study of 24 protein-encoding genes found no individual or population differences in these genes. A subsequent study of sequence variation in the seals' mitochondrial DNA also revealed low levels of genetic variation. In contrast, southern elephant seals have much higher levels of mitochondrial DNA variation. Southern elephant seals, which are found in Antarctic and sub-Antarctic waters, were also hunted, but their population size never dropped below 1000; therefore, unlike the northern elephant seals, they did not experience a genetic bottleneck.

Given enough time, all small populations will become fixed for one allele or another. Which allele becomes fixed is influenced by the initial frequencies of the alleles. If a population begins with two alleles, each with a frequency of 0.5, both alleles have an equal probability of fixation. However, if one allele is initially more common, it is more likely to become fixed.

A third effect of genetic drift is that different populations diverge genetically from one another over time. In Figure 25.14, all five populations begin with the same allelic frequency ($q = 0.5$), but because genetic drift is random, the frequencies in different populations do not change in the same way, and so populations gradually acquire genetic differences. Eventually, all the populations reach

fixation; some become fixed for one allele, and others become fixed for the alternative allele.

The three results of genetic drift (allelic frequency change, loss of genetic variation within populations, and genetic divergence between populations) take place simultaneously, and all result from sampling error. The first two results take place *within* populations, whereas the third takes place *between* populations. To see genetic drift in action, try **Animation 25.2.** **› TRY PROBLEM 37**

THINK-PAIR-SHARE Question 7

CONCEPTS

Genetic drift results from continuous small population size, founder effect (in a population established by a few founders), or a genetic bottleneck (in a population whose size has been drastically reduced). Genetic drift causes change in allelic frequencies within a population, a loss of genetic variation through the fixation of alleles, and genetic divergence between populations.

Natural Selection

A final process that brings about changes in allelic frequencies is natural selection, the differential reproduction of genotypes (see Section 24.4). Natural selection takes place when individuals with adaptive traits produce a greater number of offspring than do individuals not carrying such traits. If the adaptive traits have a genetic basis, they are inherited by the offspring and appear with greater frequency in the next generation. A trait that provides a reproductive advantage thereby increases over time, enabling populations to become better suited to their environments—that is, better adapted. Natural selection is unique among evolutionary forces in that it promotes adaptation (**Figure 25.15**).

Digital Vision/Getty Images.

25.15 Natural selection leads to adaptations, such as those seen in the polar bears that inhabit the extreme Arctic environment. These bears blend into the snowy background, which helps them in hunting seals. The hairs of their fur stay erect even when wet, and thick layers of blubber provide insulation, which protects against subzero temperatures. Their digestive tracts are adapted to a seal-based carnivorous diet.

FITNESS AND THE SELECTION COEFFICIENT

The effect of natural selection on the gene pool of a population depends on the fitness values of the genotypes in the population. **Fitness** is defined as the relative reproductive success of a genotype. Here, the term *relative* is critical: fitness is the reproductive success of one genotype compared with the reproductive successes of other genotypes in the population.

Fitness values (W) range from 0 to 1. Suppose the average number of viable offspring produced by three genotypes is as follows:

Genotypes:	A^1A^1	A^1A^2	A^2A^2
Mean number of offspring produced:	10	5	2

To calculate fitness for each genotype, we take the mean number of offspring produced by a genotype and divide it by the mean number of offspring produced by the most prolific genotype:

A^1A^1	A^1A^2	A^2A^2
$W_{11} = \frac{10}{10} = 1.0$	$W_{12} = \frac{5}{10} = 0.5$	$W_{22} = \frac{2}{10} = 0.2$

(25.17)

The fitness of genotype A^1A^1 is designated W_{11}, that of A^1A^2 is W_{12}, and that of A^2A^2 is W_{22}.

A related variable is the **selection coefficient** (s), which is the relative intensity of selection against a genotype. We usually speak of selection for a particular genotype, but keep in mind that when selection is *for* one genotype, selection is automatically *against* at least one other genotype. The selection coefficient is equal to $1 - W$, so the selection coefficients for the preceding three genotypes are

	A^1A^1	A^1A^2	A^2A^2
Selection coefficient ($1 - W$):	$s_{11} = 0$	$s_{12} = 0.5$	$s_{22} = 0.8$

CONCEPTS

Natural selection is the differential reproduction of genotypes. It is measured as fitness, which is the reproductive success of a genotype compared with that of other genotypes in a population.

✔ CONCEPT CHECK 9

The average numbers of offspring produced by three genotypes are *GG* = 6, *Gg* = 3, and *gg* = 2. What is the fitness of *Gg*?

a. 3
b. 0.5
c. 0.3
d. 0.27

THE GENERAL SELECTION MODEL

With selection, differential fitness among genotypes leads to changes in the frequencies of the genotypes over time, which, in turn, lead to changes in the frequencies of the alleles that make up the genotypes. We can predict the effect of natural selection on allelic frequencies by using a general selection model, which is outlined in Table 25.4. Use of this model requires knowledge of both the initial allelic frequencies and the fitness values of the genotypes. It assumes that mating is random and that the only force acting on a population is natural selection. The general selection model can be used to calculate the allelic frequencies after any type of selection. It is also possible to work out formulas for determining the change in allelic frequencies when selection is against recessive, dominant, or codominant traits, as well as traits in which the heterozygote has the highest fitness (Table 25.5).

TABLE 25.4 Method for determining changes in allelic frequency due to selection

	A^1A^1	A^1A^2	A^2A^2
Initial genotypic frequencies	p^2	$2pq$	q^2
Fitnesses	W_{11}	W_{12}	W_{22}
Proportional contribution of genotypes to population	p^2W^{11}	$2pqW_{12}$	q^2W_{22}
Relative genotypic frequency after selection	$\frac{p^2W_{11}}{w}$	$\frac{2pqW_{12}}{w}$	$\frac{q^2W_{22}}{w}$

Note: $w = p^2W_{11} + 2pqW_{12} + q^2W_{22}$
Allelic frequencies after selection: $p' = f(A^1) = f(A^1A^1) + {}^1/_2 f(A^1A^2)$; $q' = 1 - p'$.

TABLE 25.5 Formulas for calculating change in allelic frequencies with different types of selection

	Fitness Values			
Type of selection	A^1A^1	A^1A^2	A^2A^2	**Change in *q***
Selection against a recessive trait	1	1	1 − *s*	$\frac{-spq^2}{1-sq^2}$
Selection against a dominant trait	1	1−*s*	1−*s*	$\frac{-spq^2}{1-s+sq^2}$
Selection against a trait with no dominance	1	$1-{}^1/_2 s$	1−*s*	$\frac{-{}^1/_2 spq}{1-sq}$
Selection against both homozygotes (overdominance)	$1-s_{11}$	1	$1-s_{22}$	$\frac{pq(s_{11}p-s_{22}q)}{1-s_{11}p^2-s_{22}q^2}$

CONCEPTS

The change in allelic frequency due to selection can be determined for any type of genetic trait by using the general selection model.

WORKED PROBLEM

Let's apply the general selection model in Table 25.4 to a real example. Alcohol is a common substance in rotting fruit, where fruit-fly larvae grow and develop; larvae use the enzyme alcohol dehydrogenase (ADH) to detoxify the alcohol. In some fruit-fly populations, two alleles are present at the locus that encodes ADH: Adh^F, which encodes a form of the enzyme that migrates rapidly (fast) on an electrophoretic gel, and Adh^S, which encodes a form of the enzyme that migrates slowly on an electrophoretic gel. Female fruit flies with different *Adh* genotypes produce the following numbers of offspring when alcohol is present:

Genotype	Mean number of offspring
Adh^F/Adh^F	120
Adh^F/Adh^S	60
Adh^S/Adh^S	30

a. Calculate the fitnesses of females with these genotypes.
b. If a population of fruit flies has an initial frequency of Adh^F equal to 0.2, what will its frequency be in the next generation when alcohol is present?

Solution Strategy

What information is required in your answer to the problem?

a. The fitnesses of females of each genotype.
b. The frequency of Adh^F in the next generation when alcohol is present.

What information is provided to solve the problem?

- The mean number of offspring produced by each genotype.
- The population has an initial allelic frequency of 0.2.

Solution Steps

a. First, we must calculate the fitnesses of the three genotypes. Fitness is the relative reproductive output of a genotype and is calculated by dividing the mean number of offspring produced by that genotype by the mean number of offspring produced by the most prolific genotype. The fitnesses of the three *Adh* genotypes are as follows:

Genotype	Mean number of offspring	Fitness
Adh^F/Adh^F	120	$W_{FF} = \frac{120}{120} = 1$
Adh^F/Adh^S	60	$W_{FS} = \frac{60}{120} = 0.5$
Adh^S/Adh^S	30	$W_{SS} = \frac{30}{120} = 0.25$

b. To calculate the frequency of the Adh^F allele after selection, we can apply the table method. In the first row of the Table 25.4, we record the initial genotypic frequencies before selection has acted. If mating has been random (an assumption of the model), the genotypes will have the Hardy–Weinberg equilibrium frequencies of p^2, $2pq$, and q^2. In the second row of the Table 25.4, we put the fitness values of the corresponding genotypes. The proportion of the population represented by each genotype after selection is obtained by multiplying the initial genotypic frequency times its fitness (third row of Table 25.4). Now the genotypes are no longer in Hardy–Weinberg equilibrium.

The mean fitness (w) of the population is the sum of the proportional contributions of the three genotypes:
$(w) = p^2W_{11} + 2pqW_{12} + q^2W_{22} = 0.04 + 0.16 + 0.16 = 0.36$. The mean fitness ($w$) is the average fitness of all individuals in the population and allows the frequencies of the genotypes after selection to be obtained.

The frequency of a genotype after selection will be equal to its proportional contribution divided by the mean fitness of the population (p^2W_{11}/w for genotype

A^1A^1, $2pqW_{12}/w$ for genotype A^1A^2, and q^2W_{22}/w for genotype A^2A^2), as shown in the fourth line of Table 25.4. We can now add these values to our table as shown below:

	Adh^F/Adh^F	Adh^F/Adh^S	Adh^S/Adh^S
Initial genotypic frequencies:	$p^2 = (0.2)^2 = 0.04$	$2pq = 2(0.2)(0.8) = 0.32$	$q^2 = 0.8^2 = 0.64$
Fitnesses:	$W_{FF} = 1$	$W_{FS} = 0.5$	$W_{SS} = 0.25$
Proportional contribution of genotypes to population:	$p^2W_{FF} = (0.04)(1) = 0.04$	$2pqW_{FS} = (0.32)(0.5) = 0.16$	$q^2W_{SS} = (0.64)(0.25) = 0.16$
Relative genotypic frequency after selection:	$\frac{p^2W_{FF}}{w} = \frac{0.04}{0.36} = 0.11$	$\frac{2pqW_{FS}}{w} = \frac{0.16}{0.36} = 0.44$	$\frac{q^2W_{SS}}{w} = \frac{0.16}{0.36} = 0.44$

After the new genotypic frequencies have been calculated, the new allelic frequency of Adh^F (p') can be determined by using the now-familiar formula of Equation 25.4:

$$\begin{aligned} p' &= f(Adh^F) = f(Adh^F/Adh^F) + {}^1\!/_2 f(Adh^F/Adh^S) \\ &= 0.11 + {}^1\!/_2(0.44) = 0.33 \end{aligned}$$

and that of q' can be obtained by subtraction:

$$\begin{aligned} q' &= 1 - p' \\ &= 1 - 0.33 = 0.67 \end{aligned}$$

We predict that the frequency of Adh^F will increase from 0.2 to 0.33.

» For more practice with the selection model, try **Problem 39a** at the end of this chapter.

THE RESULTS OF SELECTION

The results of selection depend on the fitnesses of the genotypes in a population. In a population with three genotypes (A^1A^1, A^1A^2, and A^2A^2) with fitnesses W_{11}, W_{12}, and W_{22}, we can identify six different types of natural selection (Table 25.6).

TABLE 25.6 Types of natural selection

Type	Fitness Relation	Form of Selection	Result
1	$W_{11} = W_{12} > W_{22}$	Directional selection against recessive allele A^2	A^1 increases, A^2 decreases
2	$W_{11} = W_{12} < W_{22}$	Directional selection against dominant allele A^1	A^2 increases, A^1 decreases
3	$W_{11} > W_{12} > W_{22}$	Directional selection against incompletely dominant allele A^2	A^1 increases, A^2 decreases
4	$W_{11} < W_{12} < W_{22}$	Directional selection against incompletely dominant allele A^1	A^2 increases, A^1 decreases
5	$W_{11} < W_{12} > W_{22}$	Overdominance	Stable equilibrium, both alleles maintained
6	$W_{11} > W_{12} < W_{22}$	Underdominance	Unstable equilibrium

Note: W_{11}, W_{12}, and W_{22} represent the fitnesses of genotypes A^1A^1, A^1A^2, and A^2A^2, respectively.

In type 1 selection, a dominant allele A^1 confers a fitness advantage; in this case, the fitnesses of genotypes A^1A^1 and A^1A^2 are equal and higher than the fitness of A^2A^2($W_{11} = W_{12} > W_{22}$). Because both the heterozygote and the A^1A^1 homozygote have copies of the A^1 allele and produce more offspring than the A^2A^2 homozygote does, the frequency of the A^1 allele will increase over time and the frequency of the A^2 allele will decrease. This form of selection, in which one allele or trait is favored over another, is termed **directional selection**.

Type 2 selection is directional selection against a dominant allele A^1($W_{11} = W_{12} < W_{22}$). In this case, the A^2 allele increases and the A^1 allele decreases. Type 3 and type 4 selection are also directional selection, but in these cases, there is incomplete dominance, and the heterozygote has a fitness that is intermediate between the two homozygotes ($W_{11} > W_{12} > W_{22}$ for type 3; $W_{11} < W_{12} < W_{22}$ for type 4). When A^1A^1 has the highest fitness (type 3), the A^1 allele increases and the A^2 allele decreases over time. When A^2A^2 has the highest fitness (type 4), the A^2 allele increases and the A^1 allele decreases over time. Eventually, all four types of directional selection lead to fixation of the favored allele and elimination of the other allele, as long as no other evolutionary forces act on the population.

The last two types of selection (types 5 and 6) occur in special situations and lead to equilibrium, at which there is no further change in allelic frequency. Type 5 selection is referred to as **overdominance** or *heterozygote advantage*. Here, the heterozygote has higher fitness than either homozygote ($W_{11} < W_{12} > W_{22}$). With overdominance, both alleles are favored in the heterozygote, and neither allele is eliminated from the population. Initially, the allelic frequencies may change because one homozygote has higher fitness than the other; the direction of change depends on the fitness values of the two homozygotes. The allelic frequencies change with overdominant selection until a stable equilibrium is reached, at which point there is no further change. The allelic frequency at equilibrium ($\hat{q}$) depends on the fitnesses (usually expressed as selection coefficients) of the two homozygotes

$$\hat{q} = f(A^2) = \frac{s_{11}}{s_{11} + s_{22}} \tag{25.18}$$

where s_{11} represents the selection coefficient of the A^1A^1 homozygote and s_{22} represents the selection coefficient of the A^2A^2 homozygote.

An example of overdominance is sickle-cell anemia in humans, a disease that results from a mutation in one of the genes that encodes hemoglobin. People who are homozygous for the sickle-cell mutation produce only sickle-cell hemoglobin, have severe anemia, and often incur tissue damage. People who are heterozygous, with one normal copy and one mutated copy of the gene, produce both normal and sickle-cell hemoglobin, but their red blood cells contain enough normal hemoglobin to prevent sickle-cell anemia. However, heterozygotes are resistant to malaria and thus have higher fitness than do homozygotes for normal hemoglobin or homozygotes for sickle-cell hemoglobin.

The last type of selection (type 6) is **underdominance**, in which the heterozygote has lower fitness than either homozygote ($W_{11} > W_{12} < W_{22}$). Underdominance leads to an unstable equilibrium; here, allelic frequencies do not change as long as they are at equilibrium, but if they are disturbed from the equilibrium point by some other evolutionary force, they will move away from equilibrium until one allele eventually becomes fixed. › **TRY PROBLEM 40**

CONCEPTS

Natural selection changes allelic frequencies; the direction and magnitude of change depend on the intensity of selection, the dominance relations of the alleles, and the allelic frequencies. Directional selection favors one allele over another and eventually leads to fixation of the favored allele. Overdominance leads to a stable equilibrium with maintenance of both alleles in the population. Underdominance produces an unstable equilibrium because the heterozygote has lower fitness than either homozygote.

CHANGE IN THE FREQUENCY OF A RECESSIVE ALLELE DUE TO NATURAL SELECTION

The rate at which selection changes allelic frequency depends on the allelic frequency itself. If allele A^2 is lethal and recessive, $W_{11} = W_{12} = 1$, whereas $W_{22} = 0$. The frequency of the A^2 allele decreases over time (because A^2A^2 homozygotes produce no offspring), and the rate of decrease is proportional to the frequency of the allele. When the frequency of the allele is high, the change in each generation is relatively large, but as the frequency of the allele drops, a higher proportion of A^2 alleles are in heterozygous genotypes. Here, the A^2 alleles are immune to the action of natural selection because the heterozygotes have the same phenotype as the favored homozygote. Thus, selection against a rare recessive allele is very inefficient, and its removal from the population is slow.

The relation between the frequency of a recessive allele and its rate of change under natural selection has an important implication. Some people believe that the survival and reproduction of people with rare recessive genetic diseases will cause the disease gene to increase, eventually leading to degeneration of the human gene pool. This mistaken belief was the basis of eugenic laws that were passed in the early part of the twentieth century prohibiting the marriage of persons with certain genetic conditions and allowing the involuntary sterilization of others. However, most copies of rare recessive alleles are present in heterozygotes, and selection against homozygotes has little effect on the frequency of a recessive allele. There are many ethical issues associated with eugenics, but regardless, the reproduction of homozygotes for a recessive trait has little effect on the frequency of the disorder.

THINK-PAIR-SHARE Question 8

MUTATION AND NATURAL SELECTION

Recurrent mutation and natural selection act as opposing forces on detrimental alleles: mutation increases their frequency, and natural selection decreases their frequency. Eventually, these two forces reach an equilibrium in which the number of alleles added by mutation is balanced by the number of alleles removed by selection.

The frequency of a deleterious *recessive* allele at equilibrium ($\hat{q}$) is equal to the square root of the mutation rate divided by the selection coefficient:

$$\hat{q} = \sqrt{\frac{\mu}{s}} \tag{25.19}$$

The frequency of a deleterious *dominant* allele at equilibrium can be shown to be

$$\hat{q} = \frac{\mu}{s} \tag{25.20}$$

Achondroplasia is a common type of human dwarfism that results from a dominant allele. People with this condition are fertile, although they produce only about 74% as many children as produced by people without achondroplasia. The fitness of people with achondroplasia therefore averages 0.74, and the selection coefficient (s) is 1 − W, or 0.26. If we assume that the mutation rate for achondroplasia is about 3×10^{-5} (a typical mutation rate in humans), then we can predict that the equilibrium frequency for the achondroplasia allele will be

$$\hat{q} = \frac{0.00003}{0.26} = 0.0001153$$

This frequency is close to the actual frequency of the condition. **› TRY PROBLEM 43**

CONCEPTS

Mutation and natural selection act as opposing forces on detrimental alleles: mutation tends to increase their frequency, and natural selection tends to decrease their frequency, eventually producing an equilibrium.

CONNECTING CONCEPTS CONNECT

The General Effects of Forces That Change Allelic Frequencies

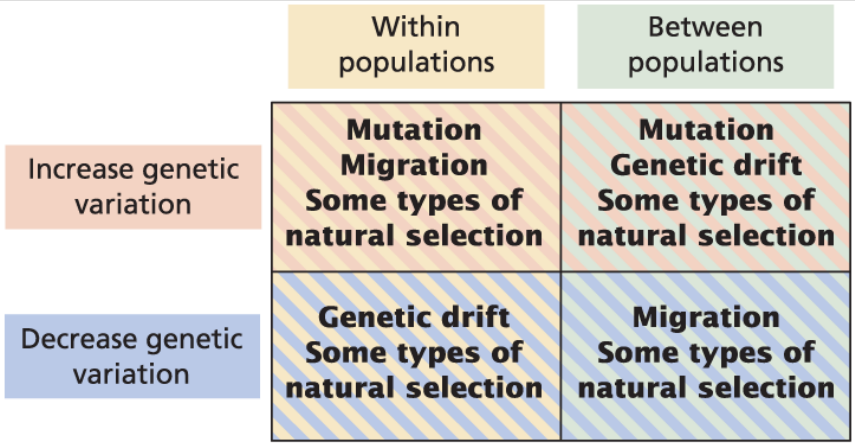
You now know that four evolutionary forces can bring about change in the allelic frequencies of a population: mutation, migration, genetic drift, and natural selection. Their short- and long-term effects on allelic frequencies are summarized in **Table 25.7**. In some cases, the change continues until one allele is eliminated and the other becomes fixed in the population. Genetic drift and directional selection eventually result in fixation, provided that these forces are the only ones acting on a population. With the other evolutionary forces, allelic frequencies change until an equilibrium point is reached, after which there is no further change in allelic frequencies. Mutation, migration, and some forms of natural selection can lead to stable equilibria.

TABLE 25.7 Effects of different evolutionary forces on allelic frequencies within populations

Force	Short-Term Effect	Long-Term Effect
Mutation	Change in allelic frequencies	Equilibrium reached between forward and reverse mutations
Migration	Change in allelic frequencies	Equilibrium reached when allelic frequencies of source and recipient populations are equal
Genetic drift	Change in allelic frequencies	Fixation of one allele
Natural selection	Change in allelic frequencies	Directional selection: fixation of one allele Overdominant selection: equilibrium reached Underdominant selection: unstable equilibrium

These evolutionary forces affect both genetic variation within populations and genetic divergence between populations. Evolutionary forces that increase genetic variation within populations are listed in the upper left quadrant of **Figure 25.16**. These forces include some types of natural selection, such as overdominance, in which

both alleles are favored. Mutation and migration also increase genetic variation within populations, because they introduce new alleles into the population. Evolutionary forces that decrease genetic variation within populations are listed in the lower left quadrant of Figure 25.16. These forces include genetic drift, which decreases variation through the fixation of alleles, and some forms of natural selection, such as directional selection.



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

25.16 Mutation, migration, genetic drift, and natural selection have different effects on genetic variation within populations and on genetic divergence between populations.

Description

The table has 2 rows and 2 columns. The columns headers, from left to right, are as follows: within populations and between populations. The row headers, from top to bottom, are as follows: increase genetic variation and decrease genetic variation. The information from the table is as follows:

Within populations, mutation, migration, and some types of natural selection increase genetic variation. Within populations, genetic drift and some types of natural selection decrease genetic variation. Between populations, mutation, genetic drift, and some types of natural selection increase genetic variation.

Between populations, migration and some types of natural selection decrease genetic variation.

These same evolutionary forces also affect genetic divergence between populations. Natural selection increases divergence between populations if different alleles are favored in different populations, but it can also decrease divergence between populations by favoring the same allele in different populations. Mutation almost always increases divergence between populations, because different mutations arise in each population. Genetic drift also increases divergence between populations, because changes in allelic frequencies due to genetic drift are random and are likely to proceed in different directions in separate populations. Migration, on the other hand, decreases divergence between populations, because it makes populations more similar in their genetic composition.

Migration and genetic drift act in opposite directions: migration increases genetic variation within populations and decreases divergence between populations, whereas genetic drift decreases genetic variation within populations and increases divergence between populations. Mutation increases both variation within populations and divergence between populations. Natural selection can either increase or decrease variation within populations, and it can increase or decrease divergence between populations.

An important point to keep in mind is that real populations are simultaneously affected by many evolutionary forces. In this chapter, we examined the effects of mutation, migration, genetic drift, and natural selection in isolation, so that the influence of each process would be clear. In the real world, however, populations are commonly affected by several evolutionary forces at the same time, and evolution results from the complex interplay of numerous processes.

Chapter 25 Review

CHAPTER SUMMARY

Section 25.1 Learning Objective: Be able to describe the gene pool of a population.

- Population genetics examines the genetic composition of groups of individuals and how their composition changes with time.
- A Mendelian population is a group of interbreeding, sexually reproducing individuals, whose set of genes constitutes the population's gene pool. Evolution takes place through changes in this gene pool.
- A population's genetic composition can be described by its genotypic and allelic frequencies.

Section 25.2 Learning Objective: Be able to explain and use the Hardy-Weinberg law.

- The Hardy–Weinberg law describes the effects of reproduction and Mendelian principles on the allelic and genotypic frequencies of a population. It assumes that a population is large; randomly mating; and free from the effects of mutation, migration, and natural selection. When these conditions are met, the allelic frequencies do not change, and the genotypic frequencies stabilize after one generation in the Hardy–

Weinberg equilibrium proportions p^2, $2pq$, and q^2, where p and q equal the frequencies of the alleles.

Section 25.3 Learning Objective: Be able to describe the effects of nonrandom mating on the gene pool of a population.

- Nonrandom mating affects the frequencies of genotypes but not those of alleles.
- Inbreeding, a type of positive assortative mating, increases the frequency of homozygotes while decreasing the frequency of heterozygotes. Inbreeding is frequently detrimental because it increases the appearance of lethal and deleterious recessive traits.

Section 25.4 Learning Objective: Be able to explain the effects of evolutionary forces (mutation, migration, genetic drift, and natural selection) on allelic frequencies of a population.

- Mutation, migration, genetic drift, and natural selection can change allelic frequencies.
- Recurrent mutation eventually leads to an equilibrium, at which allelic frequencies are determined by the relative rates of forward and reverse mutation. Change due to mutation in a single generation is usually very small because mutation rates are low.
- Migration, the movement of genes between populations, increases the amount of genetic variation within populations

and decreases the difference in allelic frequencies between populations.

- Genetic drift is change in allelic frequencies due to chance. Genetic drift arises when a population consists of a small number of individuals, is established by a small number of founders, or undergoes a major reduction in size. Genetic drift changes allelic frequencies, reduces genetic variation within populations, and causes genetic divergence among populations.
- Natural selection is the differential reproduction of genotypes; it is measured by the relative reproductive successes (fitnesses) of genotypes. The effects of natural selection on allelic frequency can be determined by applying the general selection model. Directional selection leads to the fixation of one allele. The rate of change in allelic frequency due to selection depends on the intensity of selection, the dominance relations, and the initial frequencies of the alleles.
- Mutation and natural selection can produce an equilibrium in which the number of new alleles introduced by mutation is balanced by the elimination of alleles through natural selection.

WORKED PROBLEM

A recessive allele for red hair (*r*) has a frequency of 0.2 in population I and a frequency of 0.01 in population II. A famine in population I causes a number of people in population I to migrate

to population II, where they reproduce randomly with the members of population II. Geneticists estimate that, after migration, 15% of the people in population II consist of people who migrated from population I. What will be the frequency of red hair in population II after the migration?

» Solution Strategy

What information is required in your answer to the problem?

The frequency of red hair in population II after the migration.

What information is provided to solve the problem?

- The allele for red hair (*r*) is recessive.
- The initial frequency of *r* in population I is 0.2.
- The initial frequency of *r* in population II is 0.01.
- After migration, 15% of the people in population II consist of people who migrated from population I.

For help with this problem, review:

Migration in Section 25.4.

» Solution Steps

From Equation 25.14, the allelic frequency in a population after migration (q'_{II}) is

$$q'_{\text{II}} = q_{\text{I}}(m) + q_{\text{II}}(1 - m)$$

where q_{I} and q_{II} are the allelic frequencies in population I (migrants) and population II (residents) respectively, and m is the proportion of population II that consists of migrants. In this problem, the frequency of red hair is 0.2 in population I and 0.01 in population II. Because 15% of population II consists of migrants, m = 0.15. Substituting these values into Equation 25.14, we obtain

$$q'_{\text{II}} = 0.2(0.15) + (0.01)(1 - 0.15) = 0.03 + 0.0085 = 0.0385$$

which is the expected frequency of the allele for red hair in population II after the migration. Red hair is a recessive trait; if mating is random for hair color, the frequency of red hair in population II after migration will be

$$f(rr) = q^2 = (0.0385)^2 = 0.0015$$

Recall: With random mating, the expected frequencies of the genotypes are p^2 (*RR*), $2pq$ (*Rr*), and q^2 (*rr*).

IMPORTANT TERMS

genetic rescue
Mendelian population
gene pool
genotypic frequency
allelic frequency
Hardy–Weinberg law
Hardy–Weinberg equilibrium
positive assortative mating
negative assortative mating
inbreeding
outcrossing
inbreeding coefficient
inbreeding depression
equilibrium
migration (gene flow)
sampling error
genetic drift
effective population size
founder effect
genetic bottleneck
fixation
fitness
selection coefficient
directional selection
overdominance
underdominance

ANSWERS TO CONCEPT CHECKS

1. There are fewer alleles than genotypes, so the gene pool can be described by fewer parameters when allelic frequencies are used. Additionally, the genotypes are temporary assemblages of alleles that break down each generation; the alleles are passed from generation to generation in sexually reproducing organisms.
2. a
3. c
4. 0.10
5. b
6. c
7. c
8. d
9. b

Chapter 25 Assessment

COMPREHENSION QUESTIONS

Section 25.1

1. What is a Mendelian population? How is the gene pool of a Mendelian population usually described?

Section 25.2

2. What are the predictions given by the Hardy–Weinberg law?

3. What assumptions must be met for a population to be in Hardy–Weinberg equilibrium?

4. What is random mating?

5. Give the genotypic frequencies expected under the Hardy–Weinberg law for (a) an autosomal locus with three alleles and (b) an X-linked locus with two alleles.

Section 25.3

6. Define inbreeding and briefly describe its effects on a population.

Section 25.4

7. What determines the allelic frequencies at mutational equilibrium?

8. What factors affect the magnitude of change in allelic frequencies due to migration?

9. Define *genetic drift*, and give three ways in which it can arise. What effect does genetic drift have on a population?

10. What is effective population size? How does it affect the amount of genetic drift?

11. Define *natural selection* and *fitness*.

12. Briefly describe the differences between directional selection, overdominance, and underdominance. Describe the effect of each type of selection on the allelic frequencies of a population.

13. What factors affect the rate of change in allelic frequency due to natural selection?

14. Compare and contrast the effects of mutation, migration, genetic drift, and natural selection on genetic variation within populations and on genetic divergence between populations.

APPLICATION QUESTIONS AND PROBLEMS

Section 25.1

15. How would you respond to someone who said that models are useless in studying population genetics because they represent oversimplifications of the real world?

16. *Voles (*Microtus ochrogaster*) were trapped in fields in southern Indiana and genotyped for a locus that encodes transferrin (a blood protein). The following numbers of genotypes were recorded, where T^E and T^F represent different alleles:

T^ET^E	T^ET^F	T^FT^F
407	170	17

Calculate the genotypic and allelic frequencies of the transferrin locus for this population.

Tom McHugh/Science Source.

17. DATA ANALYSIS *Jean Manning, Charles Kerfoot, and Edward Berger studied genotypic frequencies at the phosphoglucose isomerase (GPI)

locus in the cladoceran *Bosmina longirostris* (a small crustacean known as a water flea). They collected 176 of the animals from a single location in Union Bay in Seattle, Washington, and determined their GPI genotypes by using electrophoresis (J. Manning, W. C. Kerfoot, and E. M. Berger. 1978. *Evolution* 32:365–374).

Genotype	Number
S^1S^1	4
S^1S^2	38
S^2S^2	134

Determine the genotypic and allelic frequencies for this population.

18. CONNECT *Orange coat color in cats is due to an X-linked allele (X^O) that is codominant with the allele for black (X^+; see Chapter 4 for X-linked inheritance). When genotypes at the orange locus were determined for a sample of cats in Minneapolis and St. Paul, Minnesota, the following data were obtained:

X^OX^O females	11
X^OX^+ females	70

X^+X^+ females	94
X^OY males	36
X^+Y males	112

Calculate the frequencies of the X^O and X^+ alleles for this population.

19. CONNECT Assume that there are two different alleles (A^1 and A^2) in a population of autotriploid individuals (see Chapter 9 for a discussion of autotriploidy). Write equations for calculating allelic frequencies in this population.

Section 25.2

20. Use the graph shown in **Figure 25.4** to determine which genotype is most frequent when the frequency of the *A* allele is
    a. 0.2
    b. 0.5
    c. 0.8

21. A total of 6129 North American Caucasians were blood typed for the MN locus, which is determined by two codominant alleles, L^M and L^N. The following data were obtained:

Blood type	Number
M	1787
MN	3039
N	1303

Use a chi-square test to determine whether this population is in Hardy–Weinberg equilibrium at the MN locus.

22. Assume that the phenotypes of the lady beetles shown in **Figure 25.2b** are encoded by the following genotypes:

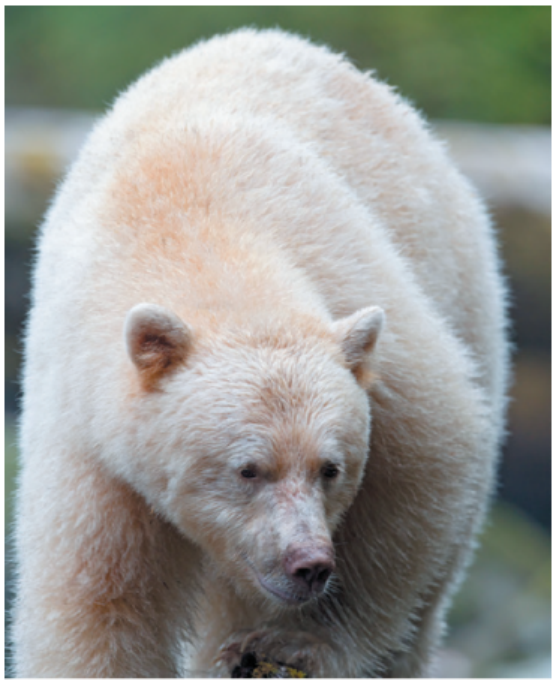
Phenotype	Genotype
All black	*BB*
Some black spots	*Bb*
No black spots	*bb*

a. For the lady beetles shown in the figure, calculate the frequencies of the genotypes and the frequencies of the alleles.
b. Use a chi-square test to determine whether the lady beetles shown are in Hardy–Weinberg equilibrium.

23. *Most black bears (*Ursus americanus*) are black or brown in color. However, occasional white bears of this species appear in some populations along the coast of British Columbia. Kermit Ritland and his colleagues determined that white coat color in these bears results from a recessive mutation (*G*) caused by a single nucleotide replacement in which guanine substitutes for adenine at the melanocortin-1 receptor locus (*mc1r*), the same locus responsible for red hair in humans (K. Ritland, C. Newton, and H. D. Marshall. 2001. *Current Biology* 11:1468–1472). The wild-type allele at this locus (*A*) encodes black or brown color. Ritland and his colleagues collected samples from bears on three islands and determined their genotypes at the *mc1r* locus:

Genotype	Number
AA	42
AG	24
GG	21

a. What are the frequencies of the *A* and *G* alleles in these bears?
b. Give the genotypic frequencies expected if the population is in Hardy–Weinberg equilibrium.
c. Use a chi-square test to compare the number of observed genotypes with the number expected under Hardy–Weinberg equilibrium. Is this population in Hardy–Weinberg equilibrium? Explain your reasoning.



Wendy Shattil/Alamy Stock Photo.

24. Genotypes of leopard frogs from a population in central Kansas were determined for a locus (*M*) that encodes the enzyme malate dehydrogenase. The following numbers of genotypes were observed:

Genotype	Number
M^1M^1	20
M^1M^2	45
M^2M^2	42
M^1M^3	4

M^2M^3	8
M^3M^3	6
Total	125

a. Calculate the genotypic and allelic frequencies for this population.
b. What would the expected numbers of genotypes be if the population were in Hardy–Weinberg equilibrium?

25. Full color (*D*) in domestic cats is dominant over dilute color (*d*). Of 325 cats observed, 194 have full color and 131 have dilute color.
    a. If this population of cats is in Hardy–Weinberg equilibrium for the dilution locus, what is the frequency of the dilute (*d*) allele?
    b. How many of the 194 cats with full color are likely to be heterozygous?

26.

*Tay–Sachs disease is an autosomal recessive disorder (see section 6.2 in Chapter 6). Among Ashkenazi Jews, the frequency of Tay–Sachs disease is 1 in 3600. If the Ashkenazi population is mating randomly with respect to the Tay–Sachs gene, what proportion of the population consists of heterozygous carriers of the Tay–Sachs allele?

27. In the plant *Lotus corniculatus*, cyanogenic glycoside protects against insect pests and even grazing by cattle. The presence of this glycoside in an individual plant is due to a simple dominant allele. A population of *L. corniculatus* consists of 77 plants that possess cyanogenic glycoside and 56 that lack the compound. What is the frequency of the dominant allele responsible for the presence of cyanogenic glycoside in this population?

28. Color blindness in humans is an X-linked recessive trait. Approximately 10% of the men in a particular population are color blind.
    a. If mating is random with respect to the color-blindness locus, what is the frequency of the color-blindness allele in this population?
    b. What proportion of the women in this population are expected to be color blind?
    c. What proportion of the women in this population are expected to be heterozygous carriers of the color-blindness allele?

Section 25.3

29. *The human MN blood type is determined by two codominant alleles, L^M and L^N. The frequency of L^M in Eskimos on a small Arctic island is 0.80.
    a. If random mating takes place in this population, what are the expected frequencies of the M, MN, and N blood types on the island?

b. If the inbreeding coefficient for this population is 0.05, what are the expected frequencies of the M, MN, and N blood types on the island?

30. Demonstrate mathematically that full-sib mating ($F = \frac{1}{4}$) reduces heterozygosity by $\frac{1}{4}$ with each generation.

Section 25.4

31. *The forward mutation rate for piebald spotting in guinea pigs is 8×10^{-5}; the reverse mutation rate is 2×10^{-6}. If no other evolutionary forces are assumed to be acting, what is the expected frequency of the allele for piebald spotting in a population that is in mutational equilibrium?

32.

For three years, Gunther Schlager and Margaret Dickie estimated the forward and reverse mutation rates for five loci in mice that encode various aspects of coat color by examining more than 5 million mice for spontaneous mutations (G. Schlager and M. M. Dickie. 1966. *Science* 151:205–206). They detected the following numbers of mutations at the *dilute* locus:

	Number of gametes examined	Number of mutations detected
Forward mutations	260,675	5

Reverse mutations	583,360	2

Calculate the forward and reverse mutation rates at this locus. If these mutation rates are representative of rates in natural populations of mice, what would the expected equilibrium frequency of *dilute* mutations be?

33. In **Figure 25.11**, each blue dot represents one copy of the *A* allele and each red dot represents one copy of the *a* allele. Calculate the frequencies of the *A* allele in population II before and after migration. Explain why the frequency of *A* in population II changed after migration.

34. In German cockroaches, curved wing (*cv*) is recessive to normal wing (cv^+). Bill, who is raising cockroaches in his dorm room, finds that the frequency of the gene for curved wings in his cockroach population is 0.6. In his friend Joe's apartment, the frequency of the gene for curved wings is 0.2. One day Joe visits Bill in his dorm room, and several cockroaches jump out of Joe's hair and join the population in Bill's room. Bill estimates that now, 10% of the cockroaches in his dorm room are individual roaches that jumped out of Joe's hair. What is the new frequency of curved wings among cockroaches in Bill's room?

35. *A population of water snakes is found on an island in Lake Erie. Some of the snakes are banded and some are unbanded; banding is caused by an autosomal allele that is recessive to an allele for no bands. The frequency of banded snakes on the

island is 0.4, whereas the frequency of banded snakes on the mainland is 0.81. One summer, a large number of snakes migrate from the mainland to the island. After this migration, 20% of the island population consists of snakes that came from the mainland.

a. If both the mainland population and the island population are assumed to be in Hardy–Weinberg equilibrium for the alleles that affect banding, what is the frequency of the allele for bands on the island and on the mainland before migration?
b. After migration has taken place, what is the frequency of the allele for the banded phenotype on the island?

36. CONNECT Sampling error leads to genetic drift. Sampling error can also occur in genetic crosses. Assume that the following cross is carried out: $Aa \times aa$. Where in this cross could sampling error occur, and how would it affect the outcome of the cross? (See Chapter 3 for a review of genetic crosses.)

37. *Pikas are small mammals that live at high elevations on the talus slopes of mountains. Most populations located on mountaintops in Colorado and Montana in North America are isolated from one another; the pikas don't occupy the low-elevation habitats that separate the mountaintops and don't venture far from the talus slopes. Thus, there is little gene flow between populations. Furthermore, each population is small in size and was founded by a small number of pikas.

A group of population geneticists proposes to study the amount of genetic variation in a series of pika populations and to compare the allelic frequencies in different populations. On the basis of the biology and distribution of pikas, predict what the population geneticists will find concerning the within- and between-population genetic variation.

38. What proportion of the populations shown in **Figure 25.14** reached fixation by generations 10, 25, and 30? How does the proportion of populations that reach fixation due to genetic drift change over time?

39. *In a large, randomly mating population, the frequency of the allele (*s*) for sickle-cell hemoglobin is 0.028. The results of studies have shown that people with the following genotypes at the beta-chain locus produce the following average numbers of offspring:

Genotype	Average number of offspring produced
SS	5
Ss	6
ss	0

a. What will the frequency of the sickle-cell allele (*s*) be in the next generation?

b. What will the frequency of the sickle-cell allele be at equilibrium?

40. *Two chromosome inversions (see Chapter 9) are commonly found in populations of *Drosophila pseudoobscura*: Standard (*ST*) and Arrowhead (*AR*). When the flies are treated with the insecticide DDT, the genotypes for these inversions exhibit overdominance, with the following fitnesses:

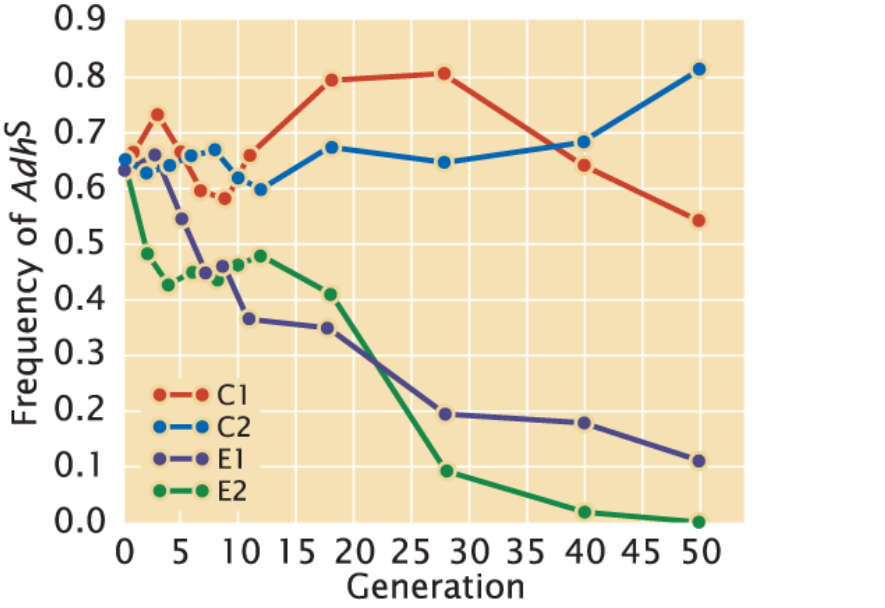
Genotype	Fitness
ST/*ST*	0.47
ST/*AR*	1
AR/*AR*	0.62

What will the frequencies of *ST* and *AR* be after equilibrium has been reached?

41. In a large, randomly mating population, the frequency of an autosomal recessive lethal allele is 0.20. What will the frequency of this allele be in the next generation if all homozygotes die before reproducing?

42. The larvae of the fruit fly *Drosophila melanogaster* normally feed on rotting fruit, which may ferment and produce high

concentrations of alcohol. Douglas Cavener and Michael Clegg studied allelic frequencies at the locus encoding alcohol dehydrogenase (*Adh*) in experimental populations of *D. melanogaster* (D. R. Cavener and M. T. Clegg. 1981. *Evolution* 35:1–10). The experimental populations were established from wild-caught flies and were raised in cages in the laboratory. Two control populations (C1 and C2) were raised on a standard cornmeal–molasses–agar diet. Two ethanol populations (E1 and E2) were raised on a cornmeal–molasses–agar diet to which 10% ethanol was added. The four populations were periodically sampled to determine the frequencies of two alleles at the alcohol dehydrogenase locus, Adh^S and Adh^F. The frequencies of the Adh^S allele in the four populations are shown in the accompanying graph.



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[Data from D. R. Cavener and M. T. Clegg. 1981. *Evolution* 35:1–10]

Description
The horizontal axis represents generation and ranges from 0 to 50, in increments of 5. The vertical axis represents frequency of big A small D small H superscript big S and ranges from 0 point 0 to 0 point 9, in increments of 0 point 1. A line representing big C 1 starts from (0, 0 point 6 8), reaches (27, 0 point 8), and ends at (50, 0 point 5 5), after many fluctuations. A line representing big C 2 starts from (0, 0 point 6 6), reaches (17, 0 point 6 8), and ends at (50, 0 point 8), after many fluctuations. A line representing big E 1 starts from (0, 0 point 6 4), reaches (27, 0 point 2), and ends at (50, 0 point 1 1), after many fluctuations. A line representing big E 2 starts from (0, 0 point 6 5), reaches (12, 0 point 4 9), and ends at (50, 0 point 0), after many fluctuations. All the lines show multiple points plotted over them. All values are approximate.

a. On the basis of these data, what conclusion might you draw about the evolutionary forces affecting the *Adh* alleles in these populations?
b. Cavener and Clegg measured the viability of the different *Adh* genotypes in the ethanol environment and obtained the following values:

Genotype	Relative viability
Adh^F / Adh^F	0.932
Adh^F / Adh^S	1.288
Adh^S / Adh^S	0.596

Using these relative viabilities, calculate fitnesses for the three genotypes. If a population has an initial frequency of $p = f$

(Adh^F) = 0.5, what is the expected frequency of Adh^F in the next generation in the ethanol environment on the basis of these fitness values?

43. *A certain form of congenital glaucoma is caused by an autosomal recessive allele. Assume that the mutation rate is 10^{-5} and that people with this condition produce, on the average, only about 80% of the offspring produced by people who do not have glaucoma.
    a. At equilibrium between mutation and selection, what will the frequency of the gene for congenital glaucoma be?
    b. What will the frequency of the disease be in a randomly mating population that is at equilibrium?

44. Examine **Figure 25.16**. Which evolutionary forces
    a. cause an increase in genetic variation both within and between populations?
    b. cause a decrease in genetic variation both within and between populations?
    c. cause an increase in genetic variation within populations, but cause a decrease in genetic variation between populations?

CHALLENGE QUESTIONS

Section 25.2

45. The frequency of allele *A* in a population is 0.8, and the frequency of allele *a* is 0.2. If the population mates randomly with respect to this locus, give all the possible matings among the genotypes at this locus and the expected proportion of each type of mating.

Section 25.4

46. The Barton Springs salamander is an endangered species found only in a few springs and wells in the city of Austin, Texas. There is growing concern that a chemical spill on a nearby freeway could pollute the springs and wipe out the species. To provide a source of salamanders to repopulate the springs in the event of such a catastrophe, a proposal has been made to establish a captive breeding population of the salamander in a local zoo. You are asked to provide a plan for the establishment of this captive breeding population, with the goal of maintaining as much of the genetic variation of the species as possible. What factors might cause loss of genetic variation in the establishment of the captive population? How could loss of such variation be prevented? With the assumption that only a limited number of salamanders can be maintained in captivity, what procedures should be instituted to ensure the long-term maintenance of as much variation as possible?

🗣️ ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 25.2

1. Miguel says that the Hardy–Weinberg law is only theoretical, of no practical value, and totally worthless because populations will never be large, randomly mating for all traits, and free from all evolutionary forces. Thus, he asserts that populations will never be in Hardy–Weinberg equilibrium. Barbara says she knows the Hardy–Weinberg law is important. Who is correct, and why?

Section 25.3

2. Name some traits for which you think humans exhibit nonrandom mating. Is mating for these traits likely to be positive or negative assortative mating? Name some traits for which you think people mate randomly. How could you test whether mating for these traits is random?

3. Assume that the frequency of an allele that causes an autosomal recessive disease is 0.03. What is the probability of a person having the disease when his or her parents are unrelated (i.e., mating is random)? What is the probability of having the disease when the parents are first cousins? What about when the parents are second cousins? (Hint: See **Table 25.3**.)

Section 25.4

4. **Figure 25.11** presents a simple mathematical model of migration. What are the assumptions of this model? Write down as many as you can think of. How reasonable are these assumptions?

5. Is migration good or bad for populations? Defend your answer.

6. What historical, social, religious, cultural, and economic factors promote genetic drift in humans? Can you think of some specific human groups in which genetic drift is likely to have occurred?

7. Examine **Figure 25.12**, which illustrates Buri's experiment on genetic drift in populations of *Drosophila*. List evidence that you see for the effects of genetic drift in the results of this experiment.

8. After the rediscovery of Mendel's principles of inheritance in the early twentieth century, eugenics became popular in many countries, including the United States. The eugenic movement proposed to improve the human species through the application of genetics. A number of eugenic laws were passed, prohibiting the marriage of people with certain conditions and allowing for the involuntary sterilization of others. Eventually this practice fell into disfavor because of ethical concerns and because many of the laws were based on bad science. Some people think that the increasing use of modern genetic techniques to test for genetic diseases and traits and to

manipulate reproductive outcomes (see Chapters 6 and 19) represents the resurgence of eugenics. What are some ethical concerns associated with encouraging the birth of people with desirable traits and discouraging the birth of those with undesirable traits?

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Introduction

1. Create a concept map that summarizes the dynamics of wolf and moose populations on Isle Royale. Include at least the following terms in your concept map: Isle Royale, wolves, moose, climate warming, Old Gray Guy, canine parvovirus, low genetic variation, inbreeding, population decline, forest decline, genetic rescue.

Sections 25.1 and 25.2

2. Draw a concept map that describes the gene pool of a population using the following concepts: Mendelian population, gene pool, genotypic frequencies, allelic frequencies, Hardy–Weinberg law, Hardy–Weinberg equilibrium, large population size, random mating, mutation, migration, natural selection.

Section 25.3

3. Use a concept map to outline the effects of nonrandom mating on the genetic makeup of a population. Include the following terms in your map: nonrandom mating, allelic frequencies, genotypic frequencies, positive assortative mating, negative assortative mating, inbreeding, outbreeding, inbreeding coefficient, inbreeding depression.

Section 25.4

4. Summarize with a concept map the effects of evolutionary forces on genetic variation within and between populations. (Hint: See **Figure 25.16**.)

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

CHAPTER 26

Evolutionary Genetics

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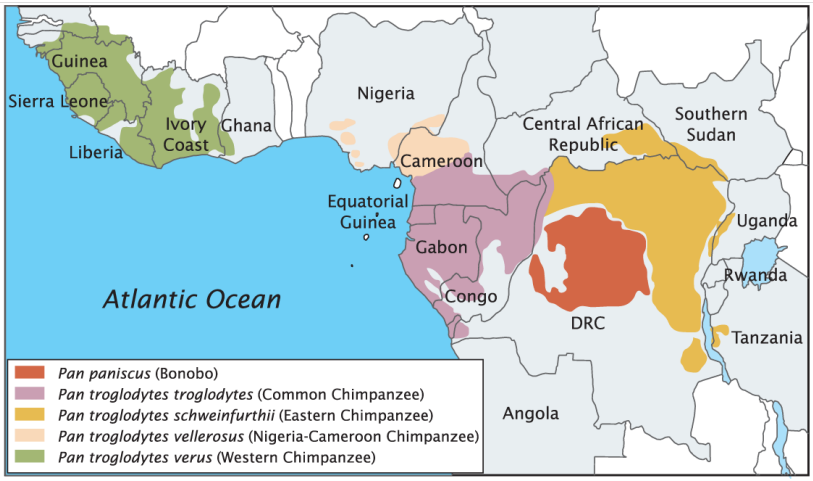
Chimpanzees and bonobos (shown here) are humans' closest living relatives. Analysis of complete genome sequences of a number of chimpanzees and bonobos indicates that extensive ancient gene flow has occurred between these two distinct species.

Chimpanzees, Bonobos, and the Question of Species

Although other hominins coexisted with modern humans (*Homo sapiens*) as recently as 40,000 years ago, today we are the only living hominin species. Our closest living relatives are chimpanzees (*Pan troglodytes*) and bonobos (*Pan paniscus*), primates that make their home in central Africa. Bonobos were recognized as different from chimpanzees in the early 1900s and were formally described as a distinct species in 1933. Bonobos are smaller and more slender than chimpanzees. They also differ socially: chimpanzees live in male-dominated groups, whereas bonobo groups are female oriented.

As we will learn in this chapter, species are often defined by the biological species concept, which proposes that a species is a group of actually or potentially interbreeding individuals that are reproductively isolated from other species. Chimpanzees and bonobos will mate in zoos, but these are artificial environments, and it has long been assumed that they do not hybridize in nature. Indeed, a number of studies have established that chimpanzees and bonobos differ in anatomy, behavior, and genetics, supporting their assignment as different species. Research has established that chimps belong to four genetically distinct clusters, described as subspecies. These groups are scattered across western and central Africa (see **Figure 26.1**). In contrast, bonobos are more uniform in their genetic makeup and are found exclusively in the Democratic Republic of the Congo.



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26.1 Bonobos and chimpanzees are found in western and central Africa.
Chimpanzees are divided into four genetically distinct subspecies. [Data from de Manuel et al., *Science* 354: 477–481, 2016.]

Description
Five species of bonobos and chimpanzees are shown in the map in the regions as follows:

Pan paniscus (Bonobo): D R C.

Pan troglodytes troglodytes (Common Chimpanzee): Cameroon, Equatorial Guniea, Gabon, Congo, Central African Republic, and D R C.

Pan troglodytes schweinfurthii (Eastern Chimpanzee): Central African Republic, D R C, Southern Sudan, Uganda, and Tanzania.

Pan troglodytes vellerosus (Nigeria-Cameroon Chimpanzee): Nigeria and Cameroon.

Pan troglodytes verus (Western Chimpanzee): Guinea, Sierra Leone, Liberia, Ivory Coast, and Ghana.

In 2016, Marc de Manuel and his colleagues undertook the most detailed study ever of genetic differences between chimpanzees and bonobos. They analyzed 75 complete genomes of chimps and bonobos from 10 different African countries. What they found was

remarkable and challenges our understanding of the biological species concept. Like previous researchers, they observed extensive genetic differences between chimpanzees and bonobos. And, consistent with prior work, they observed that chimpanzees are genetically diverse and divided into four distinct clusters. What was new and surprising was the discovery—based on the genome sequence data—of extensive ancient gene flow between chimpanzees and bonobos, gene flow that occurred after these two groups had become distinct species.

Chimpanzees and bonobos probably diverged from a common ancestor some 2.6 million years ago during the Pleistocene. The analysis by de Manuel and his colleagues suggested that after splitting into two separate species, chimps and bonobos exchanged genes from 550,000 to 100,000 years ago. The period when this mixing took place was a time of extensive climate change, when the bonobo populations were dropping in size and chimpanzee populations were expanding. Further analysis suggests that bonobo genes may have been disadvantageous in the chimpanzee genome and may have been selected against, helping to maintain the genetic distinctions that we see today between the two species. Nevertheless, central and eastern subspecies of chimpanzees today share genetic material with bonobos that is missing from the other, more distant subspecies of chimpanzees.

What is most intriguing is that human evolution is marked by similar genetic exchange among species. In its recent past, *Homo*

sapiens engaged in mixing with other species of hominins: after becoming a distinct species, modern humans interbred with at least two different species, Neanderthals and Denisovans. Evidence of this mixing is seen in present-day human genomes—some populations of *Homo sapiens* still carry within their genomes relict genes from these other hominin species (see the introductory story for Chapter 10). Gene flow among closely related species is not confined to primates: examples are found in many other types of organisms as well. As we will learn in this chapter, species are often defined as reproductively isolated groups that do not interbreed with other species. These genetic studies of bonobos, chimpanzees, and hominins challenge this traditional idea of a species as a reproductively isolated group and emphasize one of the limitations of this species definition.

THINK-PAIR-SHARE

- If chimpanzees and bonobos—considered different species—have interbred extensively in nature, does that mean the biological species concept is incorrect? What exactly is a species?
- What is the value of studying the genetics of chimpanzees and bonobos?

This chapter is about the genetic basis of evolution. Evolutionary genetics has a long history but, as illustrated by the study of chimpanzee and bonobo genomes, has been transformed in recent years by the application of powerful molecular genetic techniques. In Chapter 25, we considered the evolutionary forces that bring about change in the allelic frequencies of a population: mutation,

migration, genetic drift, and natural selection. In this chapter, we examine some specific ways in which these forces shape the genetic makeup of populations and bring about long-term evolution. We begin by looking at how the process of evolution depends on genetic variation and how genetic variation in natural populations is studied. We then turn to the evolutionary changes that result in the appearance of new species, and we see how evolutionary histories (phylogenies) can be constructed. We end the chapter by taking a look at patterns of evolutionary change at the molecular level.

26.1 Evolution Occurs Through Genetic Change Within Populations

The concept of evolution is one of the foundational principles of all of biology. Theodosius Dobzhansky, an important early leader in the field of evolutionary genetics, once remarked, “Nothing in biology makes sense except in the light of evolution.” Indeed, evolution is an all-encompassing theory that helps to make sense of much of the natural world, from the sequences of DNA found in our cells to the types of organisms that surround us. The evidence for evolution is overwhelming. Evolution has been directly observed numerous times; for example, hundreds of different insect species evolved resistance to common pesticides that were introduced after World War II. The theory of evolution is supported by the fossil record, comparative anatomy, embryology, the distribution of plants and animals (biogeography), and molecular genetics.

THINK-PAIR-SHARE Question 1

Biological Evolution

In spite of its vast importance to all fields of biology, evolution is often misunderstood and misinterpreted. In our society, the term *evolution* frequently refers to any type of change. However,

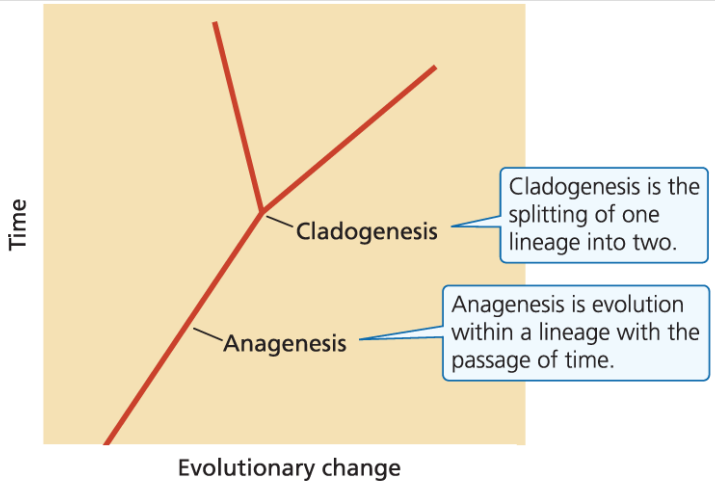
evolution, in the biological sense, refers only to a specific type of change: genetic change taking place in a group of organisms. Two aspects of this definition should be emphasized. First, biological evolution includes genetic change only. Many nongenetic changes take place in living organisms, such as the development of a complex, intelligent person from a single-celled zygote. Although remarkable, this change isn't evolution because it does not include genetic changes. Second, biological evolution takes place in *groups* of organisms. An individual organism does not evolve; what evolves is the gene pool common to a group of organisms.

Evolution as a Two-Step Process

Evolution can be thought of as a two-step process. In the first step, genetic variation arises. Genetic variation has its origin in the processes of mutation, which produces new alleles, and recombination, which shuffles alleles into new combinations. Both of these processes are random and produce genetic variation continually, regardless of evolution's requirement for it. The second step in the process of evolution is change in the frequencies of genetic variants. The various evolutionary forces discussed in Chapter 25 cause some alleles in the gene pool to increase in frequency and other alleles to decrease in frequency. This shift in the composition of the gene pool common to a group of organisms constitutes evolutionary change.

We can differentiate between two types of evolution that take place within a group of organisms connected by reproduction (Figure 26.2). **Anagenesis** refers to evolution taking place in a single lineage (a group of organisms connected by ancestry) over time. Another type of evolution is **cladogenesis**, the splitting of one lineage into two. When a lineage splits, the two branches no longer have a common gene pool and evolve independently of each other. New species arise through cladogenesis. **› TRY PROBLEM 21**

THINK-PAIR-SHARE Question 2



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26.2 Anagenesis and cladogenesis are two different types of evolutionary change. Anagenesis is change within an evolutionary lineage; cladogenesis is the splitting of lineages.

Description
The horizontal-axis represents evolutionary change and the vertical axis represents time. In the graph, a line inclines upward and forks in the middle into 2 directions forming a Y

shape. The fork is labeled with the word cladogenesis. A caption reads, “Cladogenesis is the splitting of one lineage into two.” The stalk of the Y is labeled anagenesis. A caption reads, “Anagenesis is evolution within a lineage with the passage of time.”

CONCEPTS

Biological evolution is genetic change that takes place within a group of organisms. Anagenesis is evolution that takes place within a single lineage; cladogenesis is the splitting of one lineage into two.

✔ CONCEPT CHECK 1

Briefly describe the two steps by which the process of evolution takes place.

Evolution in Bighorn Sheep

Rocky Mountain bighorn sheep (*Ovis canadensis*) are among North America’s most spectacular animals, characterized by the male’s magnificent horns, which curve gracefully back over the ears, spiraling down and back up beside the face (Figure 26.3). Two hundred years ago, bighorn sheep were numerous throughout western North America, ranging from Mexico to southern Alberta and from Colorado to California. Meriwether Lewis and William Clark reported numerous sightings of these animals in their expedition across the western United States from 1804 to 1806. Before 1900, there were an estimated 2 million bighorn sheep in

North America. But today, fewer than 70,000 of these beautiful animals remain, their numbers decimated by loss of habitat, competition from livestock, diseases carried by domesticated sheep, and hunting.

Stockbyte/Getty Images.

26.3 Microsatellite variation has been used to study the response of bighorn sheep to selective pressure on horn size due to trophy hunting.

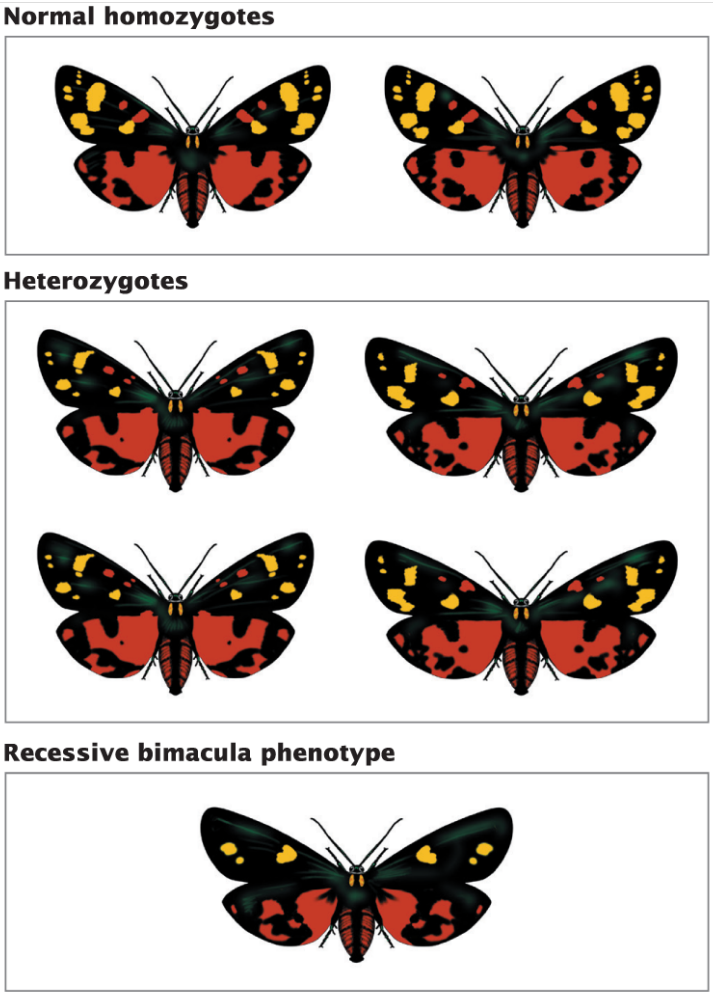
Bighorn sheep have not just decreased in number as a result of hunting; they have also evolved. Researchers have been studying the evolution of bighorn sheep in response to trophy hunting at Ram Mountain, Canada, since 1973. This population was intensively hunted until 1996, when hunting regulations increased the minimum size of the horns of rams (males) that could be shot, after which the number of rams killed by hunters each year decreased dramatically. In 2011, hunting at Ram Mountain ceased entirely. Trophy hunters selectively shoot rams with large horns, often before

they are able to reproduce, producing artificial selection for smaller horns in rams. To study the effect of this artificial selection on the population, David Coltman and his colleagues collected blood, hair, and ear samples from bighorn sheep on Ram Mountain. They extracted DNA from the samples and used PCR to amplify it. The DNA revealed variation at 20 microsatellite loci. The researchers used this variation to determine paternity in the sheep and created a detailed pedigree for the population. Using these family relationships and the quantitative genetic techniques described in Chapter 24, they were able to show that horn size in rams (as well as in females, or ewes) had moderate heritability.

The researchers observed that, in response to selective pressure imposed by the hunters, the horn length of rams decreased by almost 30% between 1973 and 1996. After hunting pressure decreased in 1996, horn length rebounded, increasing by about 13%. Through their genetic analysis, the researchers demonstrated that the decrease in horn length during the period of intensive hunting was an evolutionary response. Although a similar conclusion from an earlier study was criticized by some geneticists, who suggested that factors other than selection might account for the decrease in horn length, a 2016 analysis of the data confirmed that the evolutionary changes observed were due to selection. Ironically, the killing of trophy rams with large horns on Ram Mountain led to a decrease in the very traits prized by the hunters.

26.2 Many Natural Populations Contain High Levels of Genetic Variation

Because genetic variation must be present for evolution to take place, evolutionary biologists have long been interested in the amounts of genetic variation in natural populations and the forces that control the amount and nature of that variation. For many years, they could not examine genes directly and were limited to studying the phenotypes of organisms. Although genetic variation could not be quantified directly, studies of phenotypes suggested that many organisms within the same species harbor considerable genetic variation. Organisms in nature exhibit tremendous phenotypic variation. For example, butterflies of the species *Callimorpha* (*Panaxia*) *dominula* (**Figure 26.4**) differ in spotting patterns, frogs within the species *Lithobates* (*Rana*) *pipiens* display variation in color and spotting patterns, field mice (*Peromyscus maniculatus*) differ in the darkness of their fur, and humans vary in blood types, to mention just a few. Crosses revealed that some of these traits were inherited as simple genetic traits, but for most traits, the precise genetic basis was complex and unknown, preventing early evolutionary geneticists from quantifying the genetic variation in natural populations.



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26.4 To study genetic variation, early evolutionary geneticists were forced to rely on phenotypic traits that had a simple genetic basis. Variation in the spotting patterns of the butterfly *Callimorpha dominula* is an example.

Description

The first part labeled normal homozygotes shows two butterflies with yellow and red spots on upper wings and black spots on bottom wings. The second part labeled heterozygotes shows four butterflies with yellow and red spots on upper wings and black spots on bottom wings. The third part labeled recessive bimacula phenotype shows a butterfly with upper wings containing only a few yellow spots and bottom wings that are half black and red.

As we saw in Section 24.4, a population's response to selection depends on narrow-sense heritability, which is a measure of the additive genetic variation of a trait within a population. Many organisms respond to artificial selection carried out by humans, suggesting that the populations of these organisms contain a great deal of additive genetic variation. For example, humans have used artificial selection to produce numerous dog breeds, which vary tremendously in size, shape, color, and behavior (see Figure 24.21). Early studies of chromosome variations in *Drosophila* and plants also suggested that genetic variation in natural populations is plentiful and widespread.

Molecular Variation

In recent years, advances in molecular genetics have made it possible to investigate evolutionary change directly by analyzing protein and nucleic acid sequences. Techniques such as protein electrophoresis, analysis of microsatellite variation, and DNA sequencing have revolutionized population and evolutionary genetic studies.

ADVANTAGES OF MOLECULAR DATA

Molecular data offer a number of advantages for studying the process and patterns of evolution:

Molecular data are genetic. Evolution results from genetic change over time. Many anatomical, behavioral, and physiological traits have a genetic basis, but the relation between the underlying genes and the trait may be complex. Variation in protein and nucleic acid sequences has a clear genetic basis that is often easy to interpret.

Molecular methods can be used with all organisms. Early studies of population genetics relied on simple genetic traits—such as human blood types, banding patterns in snails, or spotting patterns in butterflies (see Figure 26.4)—which are restricted to a small collection of organisms. However, all living organisms have proteins and nucleic acids, so molecular data can be collected from any organism.

Molecular methods can be applied to a huge amount of genetic variation. An enormous amount of data can be accessed by molecular methods. The human genome, for example, contains 3.2 billion base pairs of DNA, which constitutes a large pool of information about our evolution.

All organisms can be compared using molecular data. Trying to assess the evolutionary history of distantly related organisms is often difficult because they have few characteristics in common. The evolutionary relationships between angiosperms were traditionally assessed by comparing floral anatomy, whereas the evolutionary relationships of bacteria were determined by their nutritional and staining properties. Because plants and bacteria have so few structural characteristics in common, evaluating how they are related to each other was difficult in the past. All organisms have certain molecular traits in common, such as ribosomal RNA sequences and some fundamental proteins. These molecules offer a valid basis for comparisons among all organisms.

Molecular data are quantifiable. Protein- and nucleic acid–sequence data are precise, accurate, and quantifiable, which facilitates the objective assessment of evolutionary relationships.

Molecular data often provide information about the process of evolution. Molecular data can reveal important clues about the process of evolution. For example, the results of a study of DNA sequences revealed that one type of insecticide resistance in mosquitoes probably arose from a single mutation that subsequently spread throughout the world.

The amount of molecular information is large and growing. Today, the very large collection of DNA and protein sequences that are available in publicly accessible databases can be used for making evolutionary comparisons and inferring mechanisms of evolution.

DNA SEQUENCE DATA

The rapid and inexpensive methods for sequencing DNA that are now available (see Chapter 19) allow the detection of genetic variation in almost any population. DNA sequence data often reveal processes that influence evolution, such as whether natural selection has taken place, and they are invaluable for determining the evolutionary relationships of different organisms. The use of PCR means that data can be obtained from a very small initial sample of DNA, which often facilitates studies of wild populations. For example, genetic variation in wolf populations (see the introduction to Chapter 25) has been measured and analyzed from DNA isolated from samples of wolf scat (feces).

In one example of how molecular variation has been used in evolutionary studies, Alfred Roca and his colleagues used DNA sequencing to reassess the genetic relationships among African elephants. They obtained tissue samples from 195 elephants by shooting them with needlelike darts that fell to the ground after hitting an elephant but retained a small plug of skin. From the skin samples, the scientists sequenced 1732 base pairs of DNA from four nuclear genes. Their analysis revealed large genetic differences between forest elephants and savannah elephants, suggesting that there is limited gene flow (migration) between these two groups of elephants. On the basis of these results, the scientists proposed that two different species of elephants exist in Africa.

POPULATION VARIATION

Numerous studies have demonstrated that most populations of organisms possess large amounts of variation in their protein and DNA sequences. Individuals within populations are not identical in their DNA sequences but instead differ at many nucleotide sites.

One explanation for the extensive molecular variation that is observed in natural populations is the **neutral-mutation hypothesis** (also known as the neutral theory), which proposes that much molecular variation is adaptively neutral: that is, individuals with different molecular variants have equal fitness. This hypothesis, first developed by Motoo Kimura, suggests that most variations in DNA and protein sequences are functionally equivalent. Because they are

functionally equivalent, natural selection does not differentiate between them, and their evolution is shaped largely by the random processes of genetic drift and mutation. The neutral-mutation hypothesis does not preclude the importance of natural selection; rather, it suggests that when selection occurs, it is largely directional, favoring the "best" allele while eliminating others. Thus, natural selection is viewed as an evolutionary force that largely limits variation.

Research has shown, however, that some genetic variation is maintained by natural selection. In these cases, genetic variants are not functionally equivalent; instead, they result in phenotypic effects that cause differences in reproduction (fitness differences). Selection that maintains variation is called **balancing selection**. Overdominance, in which the heterozygote has higher fitness than either homozygote (see Section 25.4), is one type of balancing selection. With overdominance, both alleles are maintained in a population because heterozygous individuals have the highest fitness and possess both alleles, so neither allele becomes fixed in the population. An example of overdominance is variation in the *β-globin* gene of humans that results in sickle-cell anemia: individuals heterozygous for an allele that causes sickle-cell anemia are resistant to malaria and thus have higher fitness than either homozygotes for the normal allele or homozygotes for the sickle-cell allele (see p. 786 in Chapter 25). Thus, both sickle-cell and normal alleles have been maintained in populations exposed to malaria.

CONCEPTS

Molecular techniques and data offer a number of advantages for evolutionary studies. The neutral-mutation hypothesis proposes that much molecular variation is neutral with regard to natural selection and is shaped largely by mutation and genetic drift. Variation within populations is also maintained by balancing selection.

✔ CONCEPT CHECK 2

Which statement about the neutral-mutation hypothesis is true?

a. All proteins are functionless.
b. Natural selection plays no role in evolution.
c. Many molecular variants are functionally equivalent.
d. All of the above

26.3 New Species Arise Through the Evolution of Reproductive Isolation

In Section 26.2, we discussed two types of evolution: anagenesis (change within a lineage) and cladogenesis (the splitting of lineages). Cladogenesis occurs through speciation, the process by which one population separates into two distinct evolutionary groups.

The term *species* literally means "kind" or "appearance"; **species** are different kinds or types of living organisms. In many cases, species differences are easy to recognize: a horse is clearly a different species than a chicken. Sometimes, however, species differences are not so clear. Some species of *Plethodon* salamanders are so similar in appearance that they can be distinguished only by looking at their proteins or genes.

The concept of a species has two primary uses in biology. First, a species is a particular type of organism to which a unique name has been given. For effective communication, biologists must use a standard set of names for the organisms that they study, and species names serve that purpose. When a geneticist talks about conducting crosses with *Drosophila melanogaster*, other biologists immediately understand which organism was used. The second use of the term *species* is in an evolutionary context: a species is considered an evolutionarily independent group of organisms.

The Biological Species Concept

What kinds of differences are required to consider two organisms different species? A widely used definition of a species is the **biological species concept**, first fully developed by evolutionary biologist Ernst Mayr in 1942. Mayr was primarily interested in the biological characteristics that are responsible for separating organisms into independently evolving units. He defined a species as a group of organisms whose members are capable of interbreeding with one another but are reproductively isolated from the members of other species. In other words, members of the same species have the biological potential to exchange genes, and members of different species cannot exchange genes. Because different species do not exchange genes, each species evolves independently.

Not all biologists adhere to the biological species concept, and there are several problems associated with it. For example, reproductive isolation, on which the biological species concept is based, cannot be determined from fossils, and in practice, it is often difficult to determine whether even living species are biologically capable of exchanging genes. There are many examples of organisms that are accepted as different species that sometimes exchange genes, or *hybridize* (see the introduction to this chapter). Furthermore, the biological species concept cannot be applied to asexually reproducing organisms, such as bacteria. In practice, many species are distinguished on the basis of phenotypic (usually anatomical)

differences. Biologists often assume that phenotypic differences represent underlying genetic differences; if the phenotypes of two organisms are quite different, then they probably cannot and do not interbreed in nature.

Because of these problems, some biologists have proposed alternative definitions for a species. For example, the *morphospecies concept* defines a species entirely on the basis of phenotypic (morphological) similarities and differences. The *phylogenetic species concept* defines a species as the smallest recognizable group that has a unique evolutionary history. In this chapter, we use the biological species concept because it is widely used and is based on reproductive differences.

Reproductive Isolating Mechanisms

The key to species differences under the biological species concept is reproductive isolation: the existence of biological characteristics that prevent genes from being exchanged between different species. Any biological factor or mechanism that prevents gene exchange is termed a **reproductive isolating mechanism**.

PREZYGOTIC REPRODUCTIVE ISOLATING MECHANISMS

Some species are separated by **prezygotic reproductive isolating mechanisms**, which prevent gametes from two different species from fusing and forming a hybrid zygote. Prezygotic reproductive isolating mechanisms can take several forms. In **ecological isolation**, members of two species do not encounter one another, and therefore do not reproduce with one another, because they have different ecological niches, living in different habitats and interacting with the environment in different ways. For example, some species of forest-dwelling birds feed and nest in the forest canopy, whereas others confine their activities to the forest floor. Because they never come into contact, these birds are reproductively isolated from one another. Other species are separated by **behavioral isolation**, differences in behavior that prevent interbreeding. Many male frogs attract females of the same species by using a unique, species-specific call. Two closely related frog species may use the same pond but never interbreed, because females are attracted only to the call of their own species.

Another type of prezygotic reproductive isolation is **temporal isolation**, in which reproduction in different species takes place at different times of the year. Some species of plants do not exchange genes because they flower at different times of the year. **Mechanical isolation** results from anatomical differences that prevent successful exchange of gametes. This type of isolation is seen in many insects, in which closely related species differ in their male and female genitalia, so that copulation between them is physically impossible. Finally, some species are separated by **gametic isolation**, in which

mating between individuals of different species may take place, but the gametes do not form zygotes. Male gametes may not survive in the female reproductive tract or may not be attracted to the female gametes. In other cases, male and female gametes meet but are too incompatible to fuse to form a zygote. Gametic isolation is seen in many plants, in which pollen from one species cannot fertilize the ovules of another species.

POSTZYGOTIC REPRODUCTIVE ISOLATING MECHANISMS

Other species are separated by **postzygotic reproductive isolating mechanisms**, in which gametes of two species may fuse and form a zygote, but there is no gene flow between the two species, either because the resulting hybrids are inviable or sterile or because reproduction breaks down in subsequent generations.

If prezygotic reproductive isolating mechanisms fail or have not yet evolved, mating between two organisms of different species may take place, and a hybrid zygote containing genes from two different species may be formed. In many cases, such species are still separated by **hybrid inviability**, in which incompatibility between the genomes of the two species prevents the hybrid zygote from developing. Hybrid inviability is seen in some groups of frogs, in which mating between different species and fertilization takes place, but the resulting embryos never complete development.

Other species are separated by **hybrid sterility**, in which hybrid embryos complete development but are sterile, so that genes are not passed between the two parental species. Donkeys and horses frequently mate and produce viable offspring—mules—but most mules are sterile, so there is no gene flow between donkeys and horses (but see Problem 48 at the end of Chapter 8).

Finally, some closely related species are capable of mating and producing viable and fertile F_1 progeny. However, genes do not flow between the two species because of **hybrid breakdown**, in which further crossing of the hybrids produces inviable or sterile offspring. For example, crosses among species of African cichlid fishes produce F_2 progeny that have lower viability than F_1 progeny or nonhybrid progeny. Hybrid breakdown is thought to be due to the breakup of gene interaction as alleles from the different species mix in different combination in the F_2 and later generations.

Many species are separated by multiple prezygotic and postzygotic reproductive isolating mechanisms. The different types of reproductive isolating mechanisms are summarized in **Table 26.1**.

THINK-PAIR-SHARE Questions 3 and 4

TABLE 26.1 Types of reproductive isolating mechanisms

Type	Characteristics

Prezygotic	Acts before a zygote has formed
Ecological	Differences in habitat; individuals do not meet
Behavioral	Differences in mating behavior prevent mating
Temporal	Reproduction takes place at different times
Mechanical	Anatomical differences prevent copulation
Gametic	Gametes are incompatible or not attracted to each other
Postzygotic	**Acts after a zygote has formed**
Hybrid inviability	Hybrid zygote does not survive to reproduction
Hybrid sterility	Hybrid is sterile
Hybrid breakdown	F_1 hybrids are viable and fertile, but F_2 are inviable or sterile

CONCEPTS

The biological species concept defines a species as a group of potentially interbreeding organisms that are reproductively isolated from the members of other species. Under this

definition, species are separated by prezygotic or postzygotic reproductive isolating mechanisms.

✔ CONCEPT CHECK 3

Which of the following is an example of postzygotic reproductive isolation?

a. Sperm of species A dies in the oviduct of species B before fertilization can take place.
b. Hybrid zygotes between species A and species B are spontaneously aborted early in development.
c. The mating seasons of species A and B do not overlap.
d. Males of species A are not attracted to the pheromones produced by the females of species B.

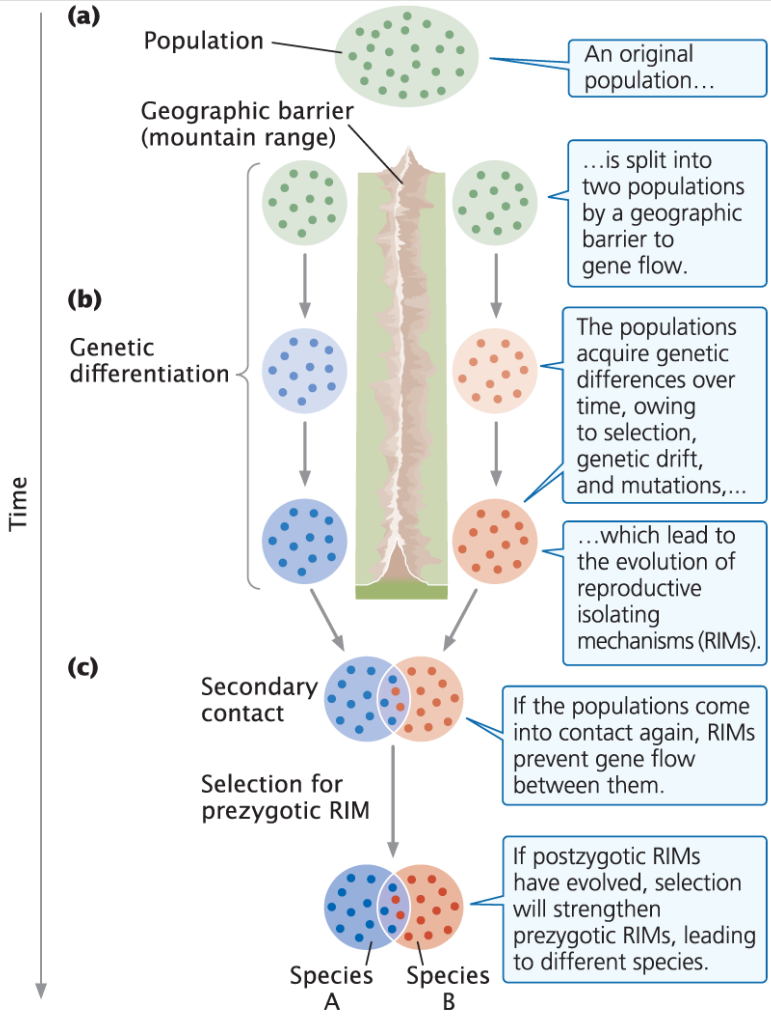
Modes of Speciation

Speciation is the process by which new species arise. In terms of the biological species concept, speciation comes about through the evolution of reproductive isolating mechanisms.

There are two principal ways in which new species arise. **Allopatric speciation** occurs when a geographic barrier splits a population into two groups and blocks the exchange of genes between them. The interruption of gene flow then leads to the evolution of genetic differences that result in reproductive isolation. **Sympatric speciation** is speciation that arises in the absence of any external barrier to gene flow; reproductive isolating mechanisms evolve within a single population.

ALLOPATRIC SPECIATION

Allopatric speciation is initiated when a geographic barrier splits a population into two or more groups and prevents gene flow between those groups (**Figure 26.5a**). Geographic barriers can take a number of forms. Uplifting of a mountain range may split a population of lowland plants into separate groups on each side of the range. Oceans serve as effective barriers for many types of terrestrial organisms, separating individuals on different islands from one another and from those on the mainland. Rivers often separate populations of fish located in separate drainages. The erosion of mountains may leave populations of alpine plants isolated on separate mountain peaks.



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26.5 Allopatric speciation is initiated by a geographic barrier to gene flow between two populations.

Description

Part “a” shows a green circle with dark green dots inside labeled population. A caption reads, “An original population is split into two populations by a geographic barrier to gene flow.” Part “b” shows a geographic barrier (mountain range) in between two populations of green circle with dark green dots on either side of the mountain range. Genetic differentiation occurs and the population on the left leads to a blue circle with dark blue dots inside, which turns darker. The population on the right leads to an orange circle with dark orange dots inside, which turns even darker. A caption reads, “The populations acquire genetic differences over time owing to selection, genetic drift, and mutations, which lead to

the evolution of reproductive isolating mechanisms (R I M's)." Part "c" shows secondary contact between the two populations. It shows a Venn diagram of the two populations. The merged region shows both orange and blue dots. A caption reads, "If the populations come into contact again, RIMs prevent gene flow between them." Selection for pre-zygotic RIM occurs and the population on the left of the Venn diagram is labeled species A, while the one on the right is labeled species B. A caption reads, "If postzygotic RIMs have evolved, selection will strengthen prezygotic RIMs, leading to different species."

After two populations have been separated by a geographic barrier that prevents gene flow between them, they evolve independently (**Figure 26.5b**). Their genetic isolation allows each population to accumulate genetic differences from the other population; these genetic differences arise through natural selection, unique mutations, and genetic drift (if the populations are small). This genetic differentiation may eventually lead to prezygotic and postzygotic reproductive isolation. It is important to note that both types of reproductive isolation arise simply as a consequence of genetic divergence.

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26.6 The Galápagos Islands are geologically young and are volcanic in origin.

The oldest islands are to the east. [Information from *Philosophical Transactions of the Royal Society of London, Series B* 351:756–772, 1996.]

Description

The map shows the following islands in decreasing order of size as follows: Isabela, Santa Cruz, Fernandina, San Salvador, San Cristobal, Santa Maria, Marchena, Espanola, Pinta, Santa Fe, Pinzon, Genovesa, Tortuga, Rabida, Los Hermanos, Champion, and Gardner.

If the geographic barrier that once separated the two populations disappears, or if individuals are able to disperse over it, the populations come into secondary contact (**Figure 26.5c**). At this point, several outcomes are possible. If limited genetic differentiation has taken place during the separation of the populations, reproductive isolating mechanisms may not have evolved or may be incomplete. Genes will flow between the two populations, eliminating any genetic differences that did arise, and the populations will remain a single species.

A second possible outcome is that genetic differentiation during separation has led to prezygotic reproductive isolating mechanisms; in this case, the two populations have become different species. A third possible outcome is that during their time apart, some genetic differentiation has taken place between the populations, leading to incompatibility in their genomes and postzygotic reproductive isolation. If postzygotic isolating mechanisms have evolved, any mating between individuals from the different populations will produce hybrid offspring that are inviable or sterile. Individuals that mate only with members of their own population will therefore have higher fitness than individuals that mate with members of the other population, so natural selection will increase the frequency of any trait that prevents interbreeding between members of the two populations. Over time, prezygotic reproductive isolating mechanisms will evolve. In short, if some postzygotic reproductive isolation exists, natural selection will favor the evolution of prezygotic reproductive isolating mechanisms to prevent wasted

reproductive effort by individuals mating with members of the other population. This process of postzygotic reproductive isolation leading to the evolution of prezygotic isolating mechanisms is termed *reinforcement*. Mathematical models of evolution support the idea that reinforcement can increase reproductive isolation. Although reinforcement is difficult to prove in natural populations, some field studies have provided evidence that reinforcement does occur in nature.

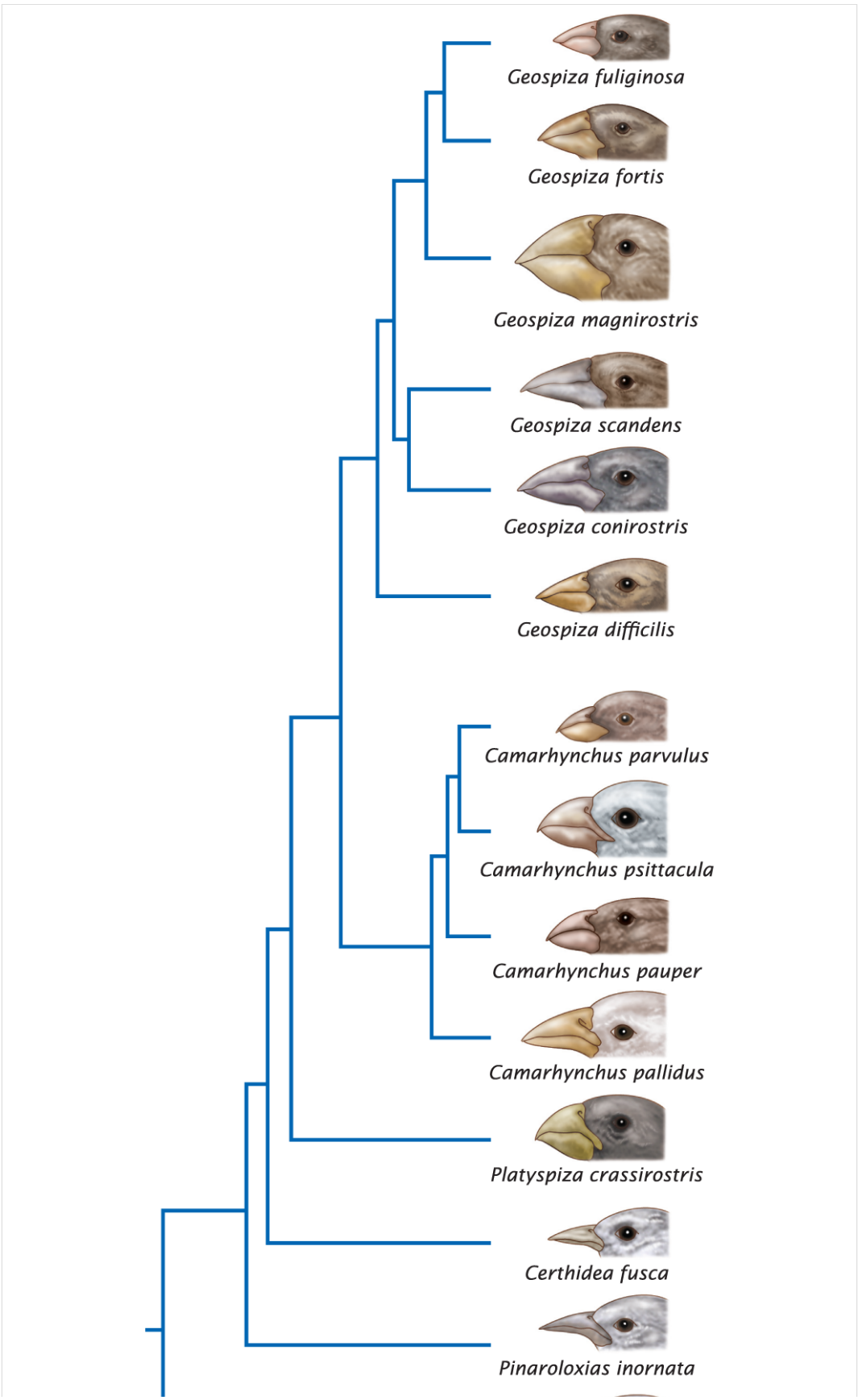
A number of variations in this general model of allopatric speciation are possible. Many new species probably arise when a small group of individuals becomes geographically isolated from the main population; for example, a few individuals of a mainland population might migrate to a geographically isolated island. In this situation, founder effect and genetic drift play a larger role in the evolution of genetic differences between the populations.

SPECIATION IN DARWIN'S FINCHES

An excellent example of allopatric speciation can be found in Darwin's finches, a group of birds that Charles Darwin observed on the Galápagos Islands during his voyage aboard the *Beagle*. The Galápagos Islands form an archipelago located about 900 km off the coast of South America (**Figure 26.6**). Consisting of more than a dozen large islands and many smaller ones, the Galápagos formed from volcanoes that erupted over a geological hot spot that has

remained stationary while the geological plate over it moved eastward during the past 3 million years. The movement of the plate pulled newly formed islands eastward, so the islands to the east (San Cristóbal and Española) are older than those to the west (Isabela and Fernandina). Over time, the number of islands in the archipelago increased as new volcanoes arose.

Darwin's finches consist of at least 14 species that are found on various islands in the Galápagos archipelago, and an additional species from Cocos Island, which is some 780 km to the north of the Galápagos (**Figure 26.7**). The birds vary in the shapes and sizes of their beaks, which are adapted for eating different types of food. Studies of the development of finch embryos have helped to reveal some of the molecular details of how differences in beak shapes have evolved (see Section 22.5). Genetic studies have demonstrated that all the finches are closely related and that they evolved from a single ancestral species that migrated to the islands from the coast of South America some 2 million to 3 million years ago. The evolutionary relationships among the 14 Galápagos species, based on studies of DNA microsatellite data, are depicted in Figure 26.7. Most of the species are separated by a behavioral isolating mechanism (song, in particular), but some of the species can and do hybridize in nature.


Geospiza fuliginosa
Geospiza fortis
Geospiza magnirostris
Geospiza scandens
Geospiza conirostris
Geospiza difficilis
Camarhynchus parvulus
Camarhynchus psittacula
Camarhynchus pauper
Camarhynchus pallidus
Platyspiza crassirostris
Certhidea fusca
Pinaroloxias inornata

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26.7 Darwin's finches evolved from a single ancestral species that migrated to the islands and underwent repeated allopatric speciation. This phylogeny is based on DNA microsatellite variation. [Data from B. R. Grant and P. R. Grant, *Bioscience* 53:965–975, 2003.]

Description
The phylogenetic tree starts with Certhidea olivacea at the bottom, branches into Pinaroloxias inornata, which further branches into Certhidea fusca. All the three show uncurved pointed beaks. Certhidea fusca further branches into Platyspiza crassirostris with a broad, stout, and curved beak. It further divides into two branches. One has Camarhynchus pallidus with a broad, long, and curved beak, which lead to Camarhynchus pauper with a small curved beak, which further leads to Camarhynchus psittacula with a big and arched beak and Camarhynchus parvulus with a short and stubby rounded beak. The other branch shows Geospiza difficilis with a sharp beak leading to two branches. One has Geospiza conirostris with a small and fine beak and Geospiza scandens with a spike-like beak, while the other has Geospiza magnirostris with a short and thick beak leading to Geospiza fortis with a small beak and Geospiza fuliginosa with short curved beak.

The first finches to arrive in the Galápagos probably colonized one of the larger eastern islands. A breeding population became established and increased over time. At some point, a few birds dispersed to another island, where they were effectively isolated from the original population, and established a new population. The new population underwent genetic differentiation owing to genetic drift and adaptation to the local conditions of the island, and it eventually became reproductively isolated from the original

population. Individual birds from the new population then dispersed to other islands and gave rise to additional species. This process was repeated many times. Occasionally, newly evolved species dispersed to an island where another species was already present, giving rise to secondary contact between the species. Today, many of the islands have more than one resident finch species.

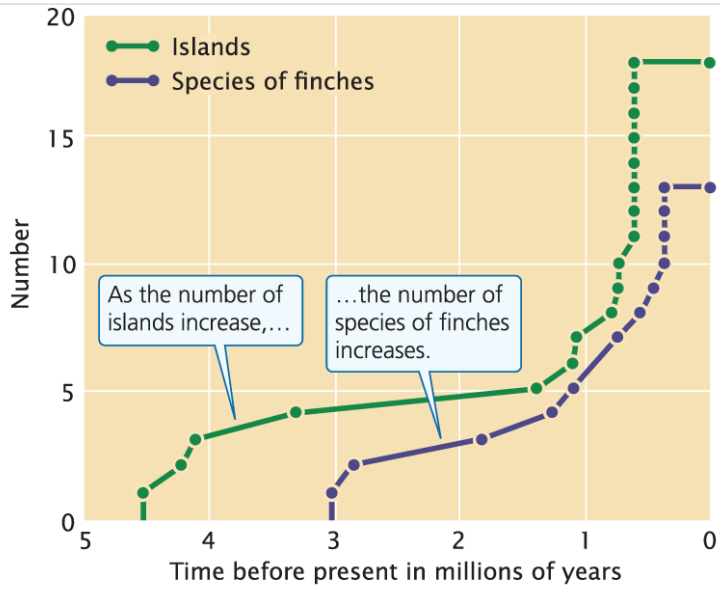
Researchers have now sequenced the entire genomes of all 14 Galápagos species and the Cocos Island species, providing a rich data set with which to interpret the history of the group. The evolutionary relationships based on these new data agreed, for the most part, with phylogenies based on earlier data (see Figure 26.7), but the genome sequences revealed some additional complexities. For example, the birds traditionally classified as *Geospiza difficilis* (the sharp-beaked ground finch), which are found on different islands, appear to comprise at least three different species. The researchers also found genetic evidence of extensive interbreeding between some species, which gave rise to species with a mix of ancestry.

The researchers also examined the DNA sequences associated with differences in beak shape and found variations at 15 chromosomal regions that were associated with differences between blunt and pointed beaks. Six of these regions contained genes that had been previously associated with cranial or facial development in birds or mammals, including the calmodulin gene discussed in Section 22.5. The strongest association was with the *Aristaless-like homeobox 1* (*ALX1*) gene, which encodes a transcription factor that plays a role

in cranial and facial development in mice and humans. Almost all birds with blunt beaks were homozygous for one haplotype at this locus, whereas most birds with sharp beaks were homozygous for a different haplotype.

The ages of the 14 finch species have been estimated with data from mitochondrial DNA. As Figure 26.8 shows, there is a strong correspondence between the number of species present at various times in the past and the number of islands in the archipelago. This correspondence is one of the most compelling pieces of evidence for the theory that the different species of finches arose through allopatric speciation.

THINK-PAIR-SHARE Question 5



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26.8 The number of species of Darwin's finches present at various times in the past corresponds with the number of islands in the Galápagos archipelago. [Data from P. R. Grant, B. R. Grant, and J. C. Deutsch, Speciation and hybridization in island birds, *Philosophical Transactions of the Royal Society of London, Series B* 351:765–772, 1996.]

Description

The horizontal-axis represents time before present in millions of years ranging from 5 at the origin to 0 with decrements of 1 and the vertical-axis represents number ranging from 0 to 20 in increments of 5. Two lines are graphed, one for islands and the other for species of finches. The line for islands started at 0 for 4 point 5 million years ago and slowly increases in the number to 5 at 1 point 3 million years ago. From 1 point 2 million to 0 point 5 million years ago the number of islands increases steeply from 5 to 18 and then becomes constant. The line for the species of finches mimics the islands line. The line starts at 3 million years ago and slowly increases until 1 million years. From 1 to 0 point 5 million years ago, the number of finches increases steeply from 5 to 14 and then becomes constant.

CONCEPTS

Allopatric speciation is initiated when a geographic barrier to gene flow splits a single population into two or more populations. Over time, the populations evolve genetic differences, which bring about reproductive isolation. After postzygotic reproductive isolating mechanisms have evolved, selection favors the evolution of prezygotic reproductive isolating mechanisms.

✔ CONCEPT CHECK 4

What role does genetic drift play in allopatric speciation?

SYMPATRIC SPECIATION

Sympatric speciation arises in the absence of any geographic barrier to gene flow; in this mode of speciation, reproductive isolating mechanisms evolve within a single interbreeding population. Sympatric speciation has long been a controversial topic within evolutionary biology. Ernst Mayr believed that sympatric speciation was impossible, and he demonstrated that many apparent cases of sympatric speciation could be explained by allopatric speciation. More recently, however, evidence has accumulated that sympatric speciation can arise, and has arisen, under special circumstances. The difficulty with sympatric speciation is that reproductive isolating mechanisms arise as a *consequence* of genetic differentiation, which takes place only if gene flow between groups is interrupted. But without reproductive isolation (or some external barrier), how can gene flow be interrupted? How can genetic differentiation arise within a single group that is freely exchanging genes?

Most models of sympatric speciation assume that genetic differentiation is initiated by selection favoring different phenotypes taking place within a single population. For example, imagine that one homozygote (A^1A^1) is strongly favored on one resource (perhaps the plant species that is host to an insect) and the other homozygote (A^2A^2) is favored on a different resource (perhaps a different host plant). Heterozygotes (A^1A^2) have lower fitness than either homozygote on both resources. In this situation, natural selection

will favor genotypes at other loci that cause positive assortative mating (matings between like individuals, see Section 25.3), so that matings between A^1A^1 and A^2A^2, which would produce A^1A^2 offspring with low fitness, will be avoided.

Now imagine that alleles at a second locus affect mating behavior, such that C^1C^1 individuals prefer mating only with other C^1C^1 individuals, and C^2C^2 individuals prefer mating with other C^2C^2 individuals. If alleles at the A locus are nonrandomly associated with alleles at the C locus so that only A^1A^1 C^1C^1 individuals and A^2A^2 C^2C^2 individuals exist, then gene flow between individuals using the different resources will be restricted, allowing the two groups to evolve further genetic differences that might lead to reproductive isolation and sympatric speciation.

The difficulty with this model is that recombination quickly breaks up the nonrandom associations between genotypes at the two loci, producing individuals such as A^1A^1 C^2C^2, which would prefer to mate with A^2A^2 C^2C^2 individuals. This mating would produce all A^1A^2 offspring, which do poorly on both resources. Thus, even limited recombination will prevent the evolution of the mating-preference genes.

CONCEPTS

Sympatric speciation arises within a single interbreeding population without any geographic barrier to gene flow.

REPRODUCTIVE ISOLATION IN APPLE MAGGOT FLIES

Sympatric speciation is more probable if the genes that affect resource utilization also affect mating preference. Such genes are indeed present in some host races—populations of specialized insect species that feed on different host plants. Researchers have studied what appear to be initial stages of speciation in host races of the apple maggot fly, *Rhagoletis pomonella* (**Figure 26.9**). Flies of this species feed on the fruits of a specific host tree. Mating takes place on and near the fruits, and the flies lay their eggs on the ripened fruits, where their larvae grow and develop. *Rhagoletis pomonella* originally existed only on the fruits of hawthorn trees, which are native to North America; 150 years ago, *R. pomonella* was first observed on cultivated apples, which are related to hawthorns but a different species. Infestations of apples by this new apple host race of *R. pomonella* quickly spread, and today many apple trees throughout North America are infested with the flies.

Joseph Berger, Bugwood.org.

26.9 Host races of the apple maggot fly (*Rhagoletis pomonella*) have evolved some reproductive isolation without any geographic barrier to gene flow.

The apple host race of *R. pomonella* probably originated when a few flies acquired a mutation that allowed them to feed on apples instead of hawthorn fruits. Because mating takes place on and near the fruits, flies that use apples are more likely to mate with other flies that use apples, leading to genetic isolation between flies using hawthorns and those using apples. Indeed, researchers found that some genetic differentiation has already taken place between the two host races. Flies lay their eggs on ripening fruit, so there has been strong selection on the flies to synchronize their reproduction with the period when the fruit of their host species is ripening. Apples ripen several weeks earlier than hawthorns. Correspondingly, the peak mating period of the apple host race is three weeks earlier than that of the hawthorn host race. This difference in the timing of reproduction between apple and hawthorn races has further reduced gene flow—to about 4%—between the two host races. Analyses of protein variation and DNA microsatellites have demonstrated that the apple and hawthorn races now differ significantly at a number of

loci. These differences have evolved in the past 150 years, and evolution appears to be ongoing.

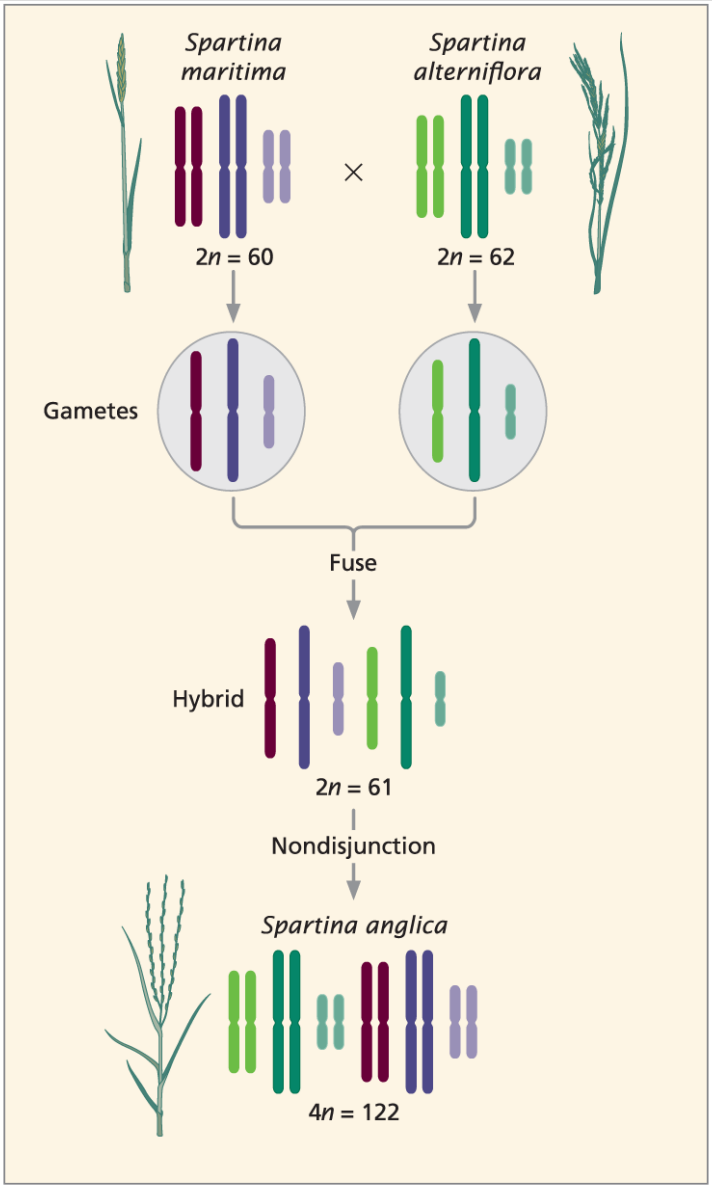
Although genetic differentiation has taken place between apple and hawthorn host races of *R. pomonella*, and some degree of reproductive isolation has evolved between them, reproductive isolation is not yet complete, and speciation has not fully taken place. ❯ **TRY PROBLEM 25**

SPECIATION THROUGH POLYPLOIDY

A special type of sympatric speciation takes place through polyploidy. Polyploid organisms have more than two sets of chromosomes (3*n*, 4*n*, 5*n*, etc.). As discussed in Section 8.4, allopolyploidy often arises when two diploid species hybridize, producing 2*n* hybrid offspring, and nondisjunction in one of the hybrid offspring then produces a 4*n* tetraploid. Because this tetraploid contains exactly two copies of each chromosome, it is usually fertile and is reproductively isolated from the two parental species by differences in chromosome number (see Figure 8.28).

Numerous species of flowering plants are allopolyploids. Speciation through polyploidy was observed when it led to a new species of salt-marsh grass that arose along the coast of England around 1870. This polyploid contains chromosomes from the European salt grass *Spartina maritima* ($2n = 60$) and the American salt grass *S. alterniflora*

(2*n* = 62; **Figure 26.10**). Seeds from the American salt grass were probably transported to England in the ballast of a ship. Regardless of how it got there, *S. alterniflora* grew in an English marsh and eventually crossed with *S. maritima*, producing a hybrid with 2*n* = 61. Nondisjunction in the hybrid then led to chromosome doubling, producing a new species, *S. anglica*, with 4*n* = 122 (see Figure 26.10). This new species subsequently spread along the coast of England.



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26.10 *Spartina anglica* arose sympatrically through allopolyploidy.

Description

The illustration starts with a cross between Spartina maritima having three pair of chromosomes (2 n equals 60) and Spartina alterniflora having three pair of chromosomes (2 n equals 62). All the chromosomes are linear. The chromosome pairs of Spartina maritime are as follows; purple long, red shorter, and violet shortest. The chromosome pairs of Spartina alterniflora are as follows; dark green long, light green shorter, and olive

green shortest. Gametes are produced from each plant having one chromosome for each of their respective colors. The gametes fuse and a hybrid is formed with six chromosomes having each color. This hybrid is labeled 2 n equals 61. Non-disjunction occurs and Spartina anglica is obtained with all the six pairs of chromosome. It is labeled 4 n equals 122.

CONCEPTS

Sympatric speciation may arise under special circumstances, such as when resource use is linked to mating preference (in host races) or when species hybridization leads to allopolyploidy.

Genetic Differentiation Associated with Speciation

As we have seen, genetic differentiation leads to the evolution of reproductive isolating mechanisms, which restrict gene flow between populations and lead to speciation. How much genetic differentiation is required for reproductive isolation to take place? This question has received considerable study by evolutionary geneticists, but unfortunately, there is no universal answer. Some newly formed species differ in many genes, whereas others appear to have undergone divergence in just a few genes.

One group of organisms that has been extensively studied for genetic differences associated with speciation is the genus *Drosophila*. The

Drosophila willistoni species complex consists of at least 12 species found in Central and South America in various stages of the process of speciation. Using analysis of genetic differences in proteins, Francisco Ayala and his colleagues genotyped flies from different geographic populations (populations that had limited genetic differences), subspecies (populations with considerable genetic differences), sibling species (newly arisen species), and nonsibling species (older species). The loci examined were used to estimate the overall degree of genetic differentiation and were not assumed to be directly involved in reproductive isolation. For each group, they computed a measure of genetic similarity, which ranges from 1 to 0 and represents the overall level of genetic differentiation (Table 26.2). They found that there was a general decrease in genetic similarity as flies evolve from geographic populations to subspecies to sibling species to nonsibling species. These data suggest that considerable genetic differentiation at many loci is required for speciation to arise. A study of *D. simulans* and *D. melanogaster*, two species that produce inviable hybrids when crossed, suggested that at least 200 genes contribute to the inviability of hybrids between the two species.

TABLE 26.2 Genetic similarity in groups of the *Drosophila willistoni* complex

Group	Mean Genetic Similarity
Geographic populations	0.970

Subspecies	0.795
Sibling species	0.517
Nonsibling species	0.352

However, other studies suggest that speciation can arise through changes in just a few genes. For example, *D. heteroneura* and *D. silvestris* are two species of Hawaiian fruit flies that exhibit behavioral reproductive isolation. The isolation is determined largely by differences in head shape: *D. heteroneura* has a hammer-shaped head with widely separated eyes that is recognized by females of the same species but rejected by *D. silvestris* females. Genetic studies indicate that only a few loci (about 10) determine the differences in head shape.

In another study, researchers examined genetic differences between two closely related species of birds, the collared flycatcher (*Ficedula albicollis*) and the pied flycatcher (*F. hypoleuca*), both of which occur in Europe. These two species are believed to have diverged from a common ancestor less than 2 million years ago, probably by allopatric speciation associated with glaciation. In areas where both species occur together, collared flycatchers and pied flycatchers sometimes mate, but the two species are separated by both pre- and postzygotic reproductive isolating mechanisms. For example, females prefer males with the plumage and song of their own

species, and hybrids do not survive as well as offspring of parents that are both from the same species.

Researchers at Uppsala University in Sweden completely sequenced the genomes of 10 male birds of each species. They found that large parts of the genomes of the two species were the same: for example, only about 5 out of 1000 nucleotides differed between species, compared with 4 out of 1000 nucleotides within species. The differences that did exist between the species were not uniform across the genome; rather, the differences tended to be concentrated in a few "divergence islands," which had sequence differences up to 50 times greater than the average for the genome. A few divergence islands were found on each chromosome, and they occurred primarily near centromeres and telomeres, suggesting that differences in chromosome structure play an important role in speciation. The divergence islands tended to show few differences within species, indicating that natural selection had favored different genes in each region.

CONCEPTS

Some newly arising species have a considerable number of genetic differences; others have few genetic differences.

26.4 The Evolutionary History of a Group of Organisms Can Be Reconstructed by Studying Changes in Homologous Characteristics

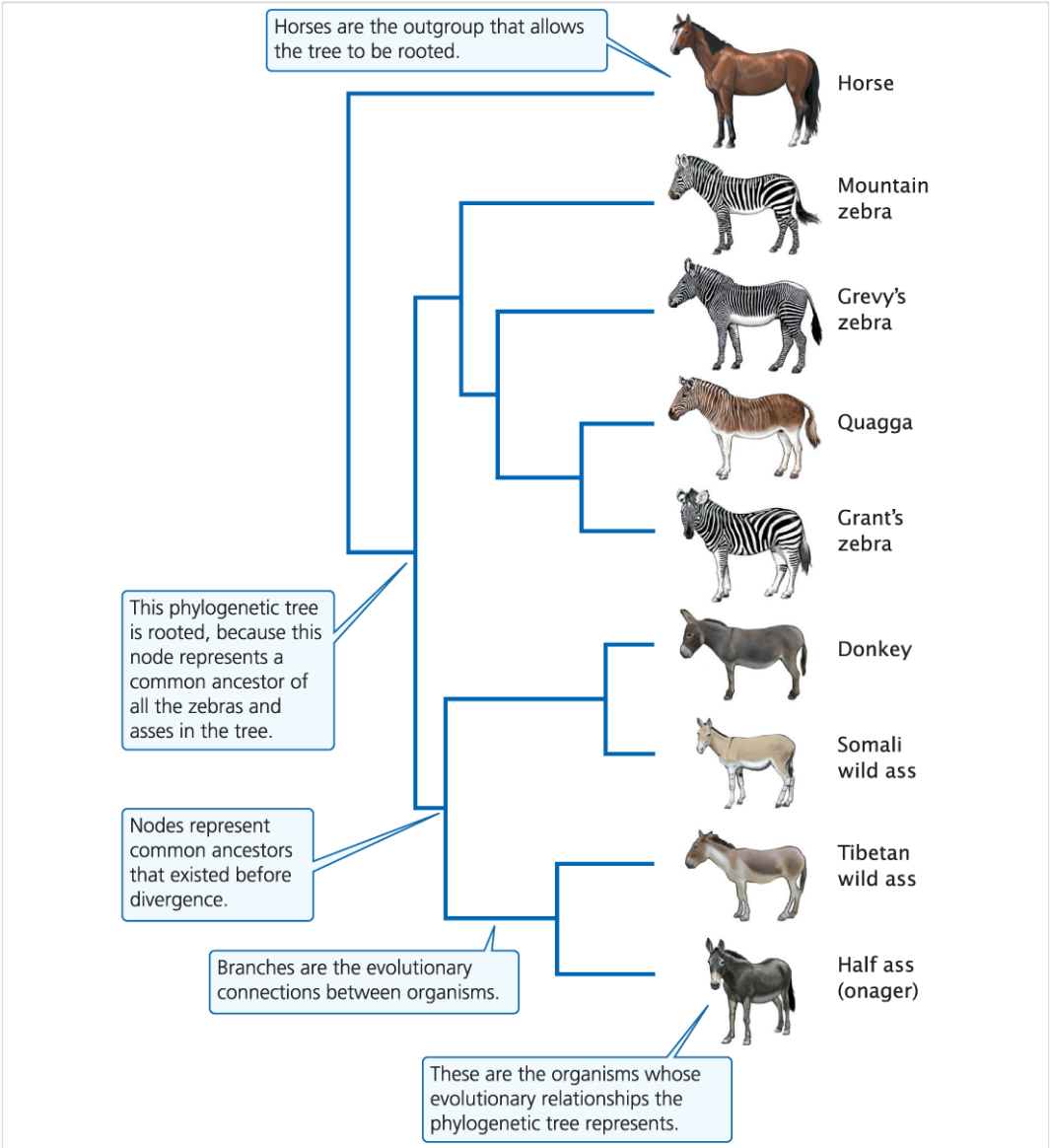
The evolutionary relationships among a group of organisms are termed a **phylogeny**. Because most evolution takes place over long periods and is not amenable to direct observation, biologists must reconstruct phylogenies by inferring the evolutionary relationships among present-day organisms. The discovery of fossils of ancestral organisms can aid in the reconstruction of phylogenies, but the fossil record is often too poor to be of much help. Thus, biologists are often restricted to analyses of characteristics in present-day organisms to determine their evolutionary relationships. In the past, phylogenies were reconstructed on the basis of phenotypic characteristics—often, anatomical traits. Today, molecular data, including protein and DNA sequences, are frequently used for this purpose.

Phylogenies are reconstructed by inferring changes that have taken place in homologous characteristics: those that have evolved from the same character in a common ancestor. For example, although the front leg of a mouse and the wing of a bat look different and have different functions, close examination of their structure and development reveals that they are indeed homologous; both evolved

from the forelimb of an early mammal that was an ancestor to both the mouse and the bat. And, because the mouse and the bat have these homologous features and others in common, we know that they are both mammals. Similarly, DNA sequences are homologous if two present-day sequences evolved from a single sequence found in an ancestor. For example, all eukaryotic organisms have a gene for cytochrome *c*, an enzyme that helps carry out oxidative respiration. This gene is assumed to have arisen in a single organism in the distant past and to have been passed down to descendants of that early ancestor. Today, all copies of the cytochrome *c* gene are homologous because they all evolved from the same original copy in the distant ancestor of all organisms that possess this gene.

A graphical representation of a phylogeny is called a **phylogenetic tree**. As shown in **Figure 26.11**, a phylogenetic tree depicts the evolutionary relationships among different organisms, in a manner similar to a pedigree that represents the genealogical relationships among family members. A phylogenetic tree consists of branches and nodes. The **branches** are the evolutionary connections between organisms. In some phylogenetic trees, the lengths of the branches represent the amount of evolutionary divergence that has taken place. The **nodes** are the points where the branches split; they represent common ancestors that existed before divergence took place. In most cases, the nodes represent past ancestors that are inferred from the analysis. When one node represents a common ancestor to all other nodes on the tree, the tree is said to be **rooted**. Trees are often rooted by including in the analysis one or more

organisms that are more distantly related to the others in the group; such a distantly related organism is referred to as an *outgroup*. For example, horses serve as the outgroup in Figure 26.11. Horses are related to zebras and asses but more distantly than zebras and asses are to each other. Some trees are unrooted and do not have a defined common ancestor for all organisms in the tree.



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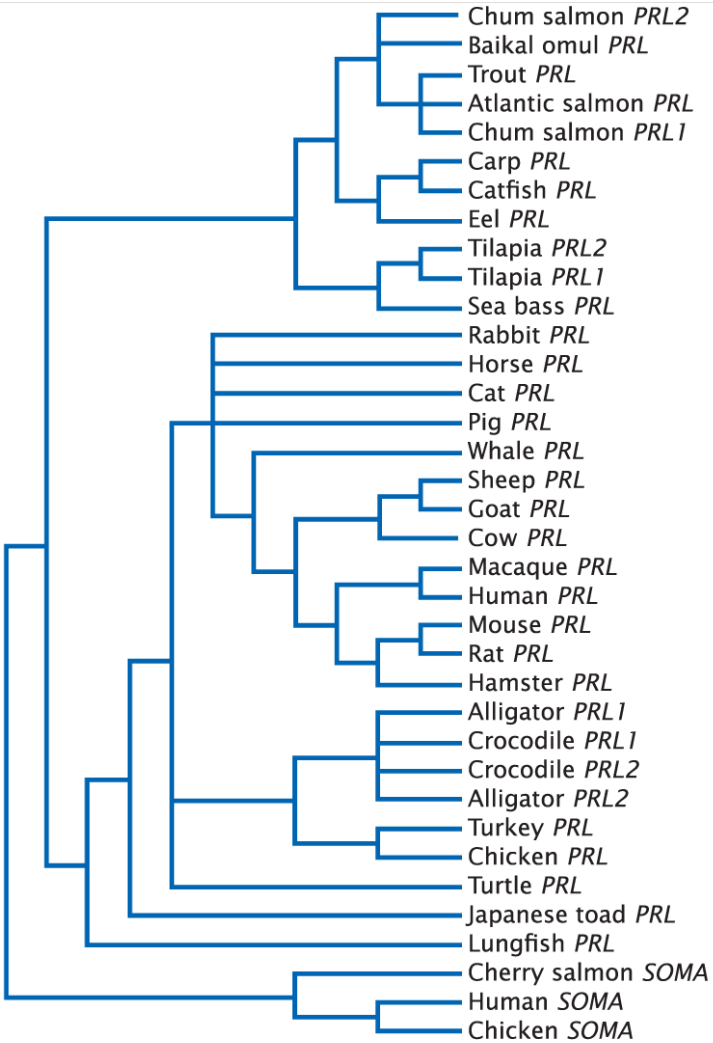
26.11 A phylogenetic tree is a graphical representation of the evolutionary relationships among a group of organisms. This phylogeny of zebras and asses is based on DNA sequences from 20,374 protein-encoding genes obtained by complete sequencing of the genomes of the species. Domesticated horses are used as an outgroup to root the tree. [Data from H. Jonsson. Speciation with gene flow in equids despite extensive chromosomal plasticity, *Proceedings of the National Academy of Sciences* 111:18655–18660, 2014.]

Description
The phylogenetic tree starts with half ass (onager) at the bottom. A caption reads, “Branches are the evolutionary connections between organisms.” The half-ass onager branches into Tibetan wild ass, which further branches into Somali wild ass and donkey. Another caption pointing towards a node in a branch reads, “Nodes represent common ancestors that existed before divergence.” The main branch further branches into two branches. A caption reads, “This phylogenetic tree is rooted, because this node represents a common ancestor of all the zebras and asses in the tree.” One branch has horse, while the other branch shows mountain zebra leading to Grevy’s zebra, which further branches into Quagga and Grant’s zebra. A caption reads, “Horses are the out group that allows the tree to be rooted.”

Phylogenetic trees are created to depict the evolutionary relationships among organisms; they are also created to depict the evolutionary relationships among DNA sequences. The latter type of phylogenetic tree is termed a **gene tree**. For example, the gene tree in **Figure 26.12** shows the evolutionary relationships among prolactin and somatropin genes in vertebrates. Because of gene duplications, multiple copies of the genes appear in some organisms. ❯ **TRY PROBLEM 31**



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26.12 A gene tree can be used to represent the evolutionary relationships among a group of genes. This gene tree is a rooted tree. *PRL* represents a prolactin gene; *PRL1* and *PRL2* are two different prolactin genes found in the same organism; and *SOMA* represents a somatropin gene, which is related to prolactin genes. [Data from M. P. Simmons and J. V. Freudestein, Uninode coding vs. gene tree parsimony for phylogenetic reconstruction using duplicate genes, *Molecular Phylogenetics and Evolution* 23:488, 2002.]

Description

The tree starts with chicken SOMA at the bottom branching to human SOMA, further branching to cherry salmon SOMA. From cherry salmon, a main branch divides into 2

branches. One branch shows sea bass P R L leading to Tilapia P R L 1 and Tilapia P R L 2. This branch leads to another two branches; one has Eel P R L, catfish P R L, and carp P R L. The other branch leads to Chum salmon P R L 2 and Baikal omul P R L leading to Trout P R L, Atlantic salmon P R L, and Chum salmon P R L 1. The second main branch shows lungfish P R L, Japanese toad P R L, and turtle P R L. From turtle P R L, two branches arise. The first branch further shows two divisions as follows: Turkey P R L and Chicken P R L and Alligator P R L 1, Crocodile P R L 1, Crocodile P R L 2, and Alligator P R L 2. The second branch shows Rabbit P R L, Horse P R L, Cat P R L, pig P R L, which leads to whale P R L. It further divides into two branches. One having Sheep P R L, Goat P R L, and Cow P R L, while the other having Macaque P R L, Human P R L, Mouse P R L, Rat P R L, and Hamster P R L.

CONCEPTS

A phylogeny represents the evolutionary relationships among a group of organisms. It is often depicted graphically by a phylogenetic tree, which consists of nodes representing the organisms and branches representing their evolutionary connections.

✔ CONCEPT CHECK 5

Which of the following features is found in a rooted tree but not in an unrooted tree?

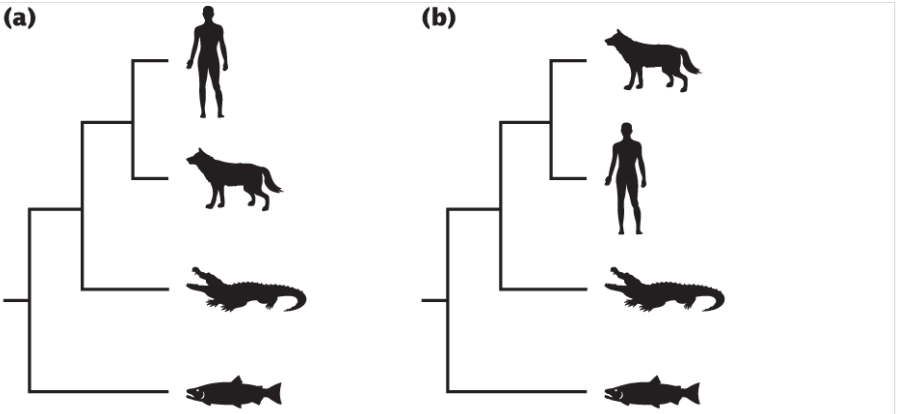
a. Nodes
b. Branches
c. A common ancestor to all other nodes
d. Branch lengths that represent the amount of evolutionary divergence between nodes

Interpreting Phylogenetic Trees

Phylogenetic trees can be represented in several different ways, which sometimes creates confusion. The organisms in the tree can

also be flipped or rotated around a node without changing their evolutionary relationships. For example, the wolf and human in **Figure 26.13a** can be rotated about their common ancestor, producing **Figure 26.13b**, which is the same phylogeny (same evolutionary relationships) as that in Figure 26.13a. What this means is that whether two organisms lie adjacent to each other in the phylogenetic tree does not necessarily indicate a close evolutionary relationship. You might assume, looking at Figure 26.13a, that wolves are more closely related to alligators than humans are to alligators, because wolves and alligators are physically next to each other in the tree. However, wolves and humans are equally related to alligators, which is clear when you flip wolves and humans around their node (Figure 26.13b).

Evolutionary relatedness is based on common ancestry. Two organisms are more closely related when they share a *more recent* common ancestor. For example, consider Figure 26.13a. Which organisms are more closely related: alligators and fish, or alligators and humans? You might, at first, be tempted to say alligators and fish, because the alligator and fish lie next to each other in the phylogenetic tree. In fact, humans and alligators are more closely related, because their most recent common ancestor occurred later in time and is therefore more recent than the common ancestor of alligator and fish (**Figure 26.14a**). This is more obvious in **Figure 26.14b**, where alligators, humans, and wolves are rotated about the node that represents their common ancestor.



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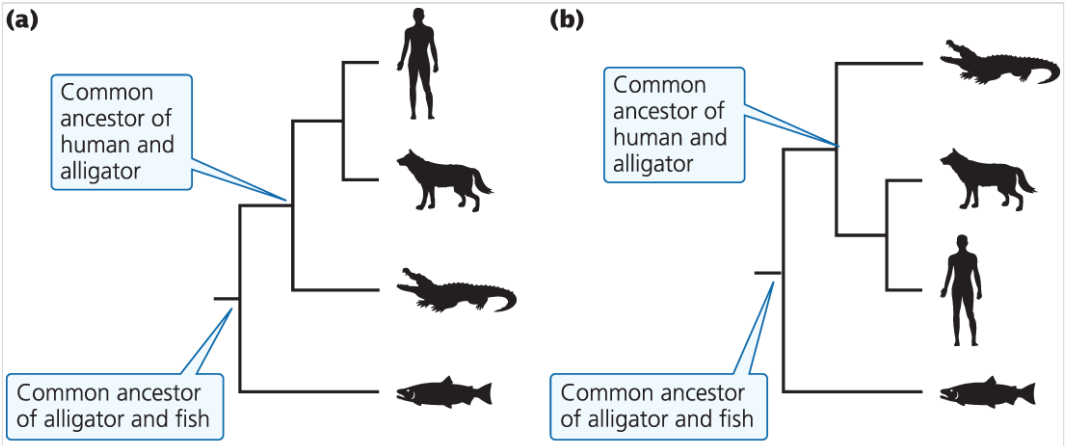
26.13 Phylogenetic trees can be depicted in different ways. The trees in parts (a) and (b) are equivalent and represent the same evolutionary relationships.

Description

Part “a” shows a main branch dividing into two branches. The first branch shows a fish at the bottom and the second branch further divides into two branches; one shows crocodile and the other shows dog at the bottom and human on top. Part “b” shows a similar phylogenetic tree, but the position of dog and human is interchanged.



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26.14 Relatedness in a phylogenetic tree is based on common ancestry: more closely related organisms are those that share a more recent common ancestor. In this tree, humans and alligators are more closely related than fish

and alligators. [Based on an example provided in D. A. Baum, S. D. Smith, and S. S. S. Donovan, The tree-thinking challenge, *Science* 310:979–981, 2005].

Description
Part “a” shows a main branch dividing into two branches. A caption pointing towards the main branch reads, “Common ancestor of alligator and fish.” The first branch shows a fish at the bottom and the second branch further divides into two branches. A caption reads, “Common ancestor of human and alligator.” One branch shows crocodile and the other shows dog at the bottom and human on top. Part “b” shows a similar phylogenetic tree and similar captions, but the position of dog and human is interchanged.

The Alignment of Homologous Sequences

Today, phylogenetic trees are often constructed from DNA sequence data. This construction requires that homologous sequences be compared. Thus, a first step is to identify homologous genes and properly align their nucleotide bases. Consider the following short sequences that might be found in two different organisms (most actual sequences used would be much longer):

Nucleotide position	1 2 3 4 5 6 7 8
Gene *X* from species A	5′ – A T T G C G A A – 3′
Gene *X* from species B	5′ – A T G C C A A C – 3′

Description
Gene X from species A at the top reads, 5 prime A T T G C G A A 3 prime. Gene X from species B below reads, 3 prime A T G C C A A C 3 prime. The nucleotides positions in both the genes are labeled 1 through 8 from left to right.

These two sequences can be aligned in several possible ways. We might assume that there have been base substitutions at positions 3, 4, 6, and 8:

Nucleotide position	1 2 3 4 5 6 7 8
Gene *X* from species A	5′ – A T T G C G A A – 3′
Gene *X* from species B	5′ – A T G C C A A C – 3′

Description
Gene X from species A at the top reads, 5 prime A T T G C G A A 3 prime. Gene X from species B below reads, 3 prime A T G (highlighted in red) C (highlighted in red) C A (highlighted in red) A C 3 prime. The nucleotides positions in both the genes are labeled 1 through 8 from left to right.

Alternatively, we might assume that a nucleotide at position 3 has been inserted or deleted, generating a gap in the sequence of species B, and that there has been a single nucleotide substitution at position 6:

Nucleotide position	1 2 3 4 5 6 7 8
Gene *X* from species A	5′ – A T T G C G A A – 3′
Gene *X* from species B	5′ – A T – G C C A A C – 3′

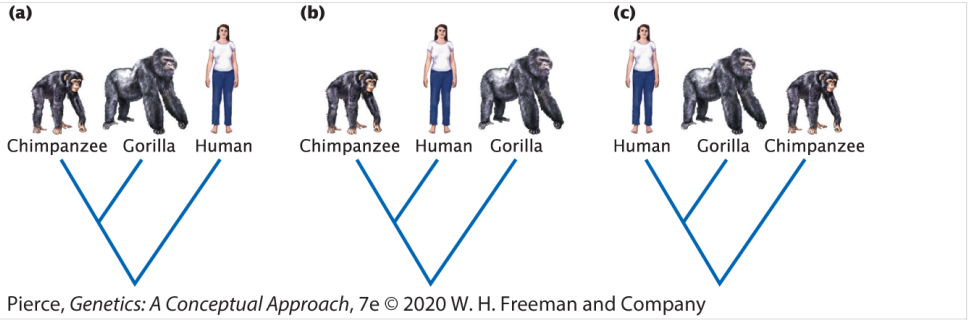
Description
Gene X from species A at the top reads, 5 prime A T T G C G A A 3 prime. Gene X from species B below reads, 3 prime A T blank G C C (highlighted in red) A A C 3 prime. The nucleotides positions in both the genes are labeled 1 through 8 from left to right.

The second alignment requires fewer evolutionary steps (a deletion or insertion plus one base substitution) than does the first alignment (four base substitutions).

Sequence alignments are usually made by computer programs that include assumptions about which types of change are more likely to take place. If two sequences have undergone a great deal of divergence, generating alignments can be difficult.

The Construction of Phylogenetic Trees

Consider a simple phylogeny that depicts the evolutionary relationships among three organisms: humans, chimpanzees, and gorillas. Charles Darwin originally proposed that chimpanzees and gorillas were closely related to humans, and modern research supports a close relationship between these three species. There are three possible phylogenetic trees for humans, chimpanzees, and gorillas (**Figure 26.15**). The goal of the evolutionary biologist is to determine which of the trees is correct. Molecular data applied to this question strongly suggest a close relationship between humans and chimpanzees.



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26.15 There are three possible phylogenetic trees for humans, chimpanzees, and gorillas.

Description

Phylogenetic tree “a” shows chimpanzees and gorillas sharing an internal node and then their ancestor sharing a node with humans. Phylogenetic tree “b” shows chimpanzees and humans sharing an internal node and then their ancestor sharing a node with gorillas. Phylogenetic tree “c” shows humans and gorillas sharing an internal node and then their ancestor sharing a node with chimpanzees.

To understand the difficulty in constructing phylogenetic trees, let’s consider for a moment the number of possible trees that might exist for a group of organisms. The number of possible rooted trees for a group of organisms is

$$\text{number of rooted trees} = \frac{(2N-3)!}{2^{N-2}(N-2)!}$$

where N equals the number of organisms included in the phylogeny, and the ! symbol stands for factorial, the product of all the integers from N to 1. Substituting values of N into this equation, we find the following numbers of possible rooted trees:

Number of organisms included in phylogeny (*N*)	Number of rooted trees
2	1
3	3
4	15
5	105
10	34,459,425
20	8.2×10^{21}

As the number of organisms in the phylogeny increases beyond just a few, the number of possible rooted trees becomes astronomically large. Clearly, choosing the best tree by directly comparing all the possibilities is impossible.

There are several different approaches to inferring evolutionary relationships and constructing phylogenetic trees. In one approach, termed the *distance approach*, evolutionary relationships are inferred on the basis of the overall degree of similarity between organisms. Typically, a number of different phenotypic characteristics or gene sequences are examined, and the organisms are grouped on the basis of their overall similarity, taking into consideration all the examined characteristics and sequences. A second approach, called

the *maximum parsimony approach*, infers phylogenetic relationships on the basis of the fewest number of evolutionary changes that must have taken place since the organisms last had an ancestor in common. A third approach, called *maximum likelihood* and *Bayesian methods*, infers phylogenetic relationships on the basis of which phylogeny maximizes the probability of obtaining the set of characteristics exhibited by the organisms. In this approach, a phylogeny with a higher probability of producing the observed characteristics in the organisms studied is preferred over a phylogeny with a lower probability. Maximum likelihood and Bayesian methods incorporate models of how evolutionary change takes place. For example, they incorporate the probability that one particular DNA base will be substituted for a different base.

With all three approaches to constructing phylogenies, several different numerical methods are available for the construction of phylogenetic trees. All include certain assumptions that help limit the number of different trees that must be considered; most rely on computer programs that compare phenotypic characteristics or sequence data to sequentially group organisms in the construction of a tree.

CONCEPTS

Molecular data can be used to infer phylogenies (evolutionary histories) of groups of organisms. The construction of phylogenies requires the proper alignment of homologous DNA sequences. Several different approaches are used to reconstruct phylogenies, including

distance methods, maximum parsimony methods, and maximum likelihood and Bayesian methods.

26.5 Patterns of Evolution Are Revealed by Molecular Changes

Our ability to analyze genetic variation at the molecular level has revealed a number of evolutionary processes and features that were formerly unsuspected. This section considers several aspects of evolution at the molecular level.

Rates of Molecular Evolution

Findings from molecular studies of numerous genes have demonstrated that different genes, and even different parts of the same gene, may evolve at different rates.

RATES OF NUCLEOTIDE SUBSTITUTION

Rates of evolutionary change in nucleotide sequences are usually measured as the rate of nucleotide substitution, which is the number of substitutions taking place per nucleotide site per year within a population. To calculate the rate of nucleotide substitution, we begin by looking at homologous sequences from different organisms. We first align the homologous sequences, and then compare the sequences and determine the number of nucleotides that differ between the two sequences. We might compare the growth-hormone sequences for mice and rats, which diverged from a common ancestor some 15 million years ago. From the number of nucleotide differences between their growth-hormone genes, we compute the number of nucleotide substitutions that must have taken place since they diverged. Because the same site may have mutated more than once, the number of nucleotide substitutions is larger than the number of nucleotide differences between two sequences; special mathematical methods have been

developed for inferring the actual number of substitutions likely to have taken place.

When we have the number of nucleotide substitutions per nucleotide site, we can use the total amount of evolutionary time that separates the two organisms (usually obtained from the fossil record) to obtain an overall rate of nucleotide substitution. For the mouse and rat growth-hormone genes, the overall rate of nucleotide substitution is approximately 8×10^{-9} substitutions per site per year.

RATES OF NONSYNONYMOUS AND SYNONYMOUS SUBSTITUTION

Nucleotide changes in a gene that alter the amino acid sequence of a protein are referred to as nonsynonymous substitutions. Nucleotide changes, particularly those at the third position of a codon, that do not alter the amino acid sequence of a protein are called synonymous substitutions. The rate of nonsynonymous substitution varies widely among mammalian genes. The rate for the α-actin protein is only 0.01×10^{-9} substitutions per site per year, whereas the rate for interferon γ is 2.79×10^{-9}, almost 300 times higher. The rate of synonymous substitution also varies among genes, but not as much as the nonsynonymous rate. For most protein-encoding genes, the rate of synonymous substitution is considerably higher than the nonsynonymous rate (Table 26.3) because synonymous mutations have little or no effect on fitness—that is, they are selectively neutral. Nonsynonymous mutations, on the other hand, alter the amino acid sequence of the protein and, in many cases, are detrimental to the fitness of the organism; most of these mutations are eliminated by natural selection.

THINK-PAIR-SHARE Question 6

TABLE 26.3 Rates of nonsynonymous and synonymous substitutions in

mammalian genes based on human–rodent comparisons

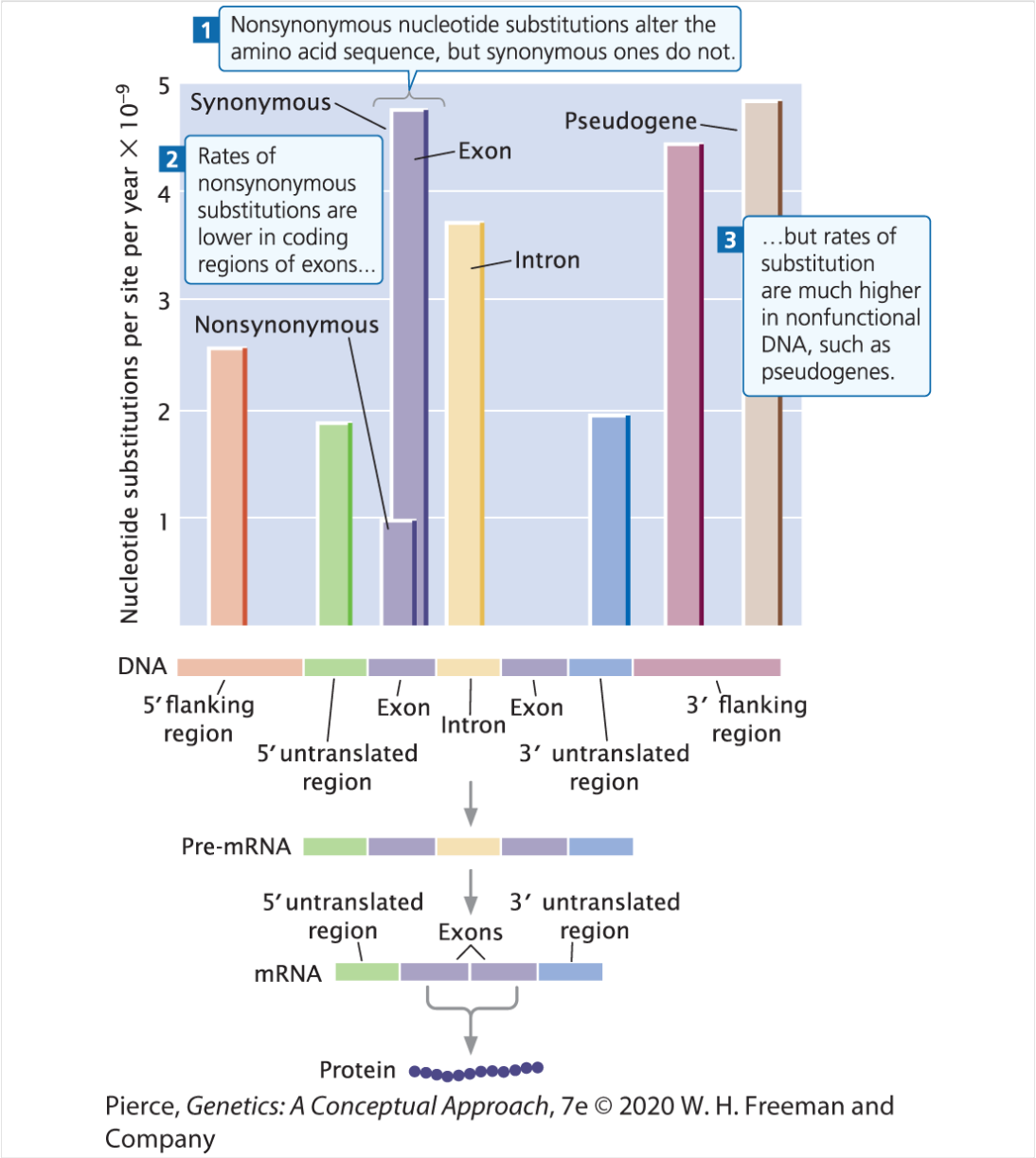
Gene	Nonsynonymous Rate (per site per 10^9 years)	Synonymous Rate (per site per 10^9 years)
α-Actin	0.01	3.68
β-Actin	0.03	3.13
Albumin	0.91	6.63
Aldolase A	0.07	3.59
Apoprotein E	0.98	4.04
Creatine kinase	0.15	3.08
Erythropoietin	0.72	4.34
α-Globin	0.55	5.14
β-Globin	0.80	3.05
Growth hormone	1.23	4.95
Histone 3	0.00	6.38
Immunoglobulin heavy chain (variable region)	1.07	5.66
Insulin	0.13	4.02
Interferon α1	1.41	3.53
Interferon γ	2.79	8.59
Luteinizing hormone	1.02	3.29
Somatostatin-28	0.00	3.97

Information from W. Li and D. Graur, *Fundamentals of Molecular Evolution* (Sunderland, Mass.: Sinauer Associates, 1991), p. 69, Table 1.

SUBSTITUTION RATES FOR DIFFERENT PARTS OF A GENE

Different parts of a gene also evolve at different rates. The highest rates of substitution occur in those regions of the gene that have the least effect on function, such as the third position of a codon, flanking regions, and introns (Figure 26.16). The 5′ and 3′ flanking regions of genes are not transcribed into RNA; therefore, substitutions in these regions do not alter the amino acid

sequence of the protein, although they may affect gene expression (see Chapters 16 and 17). Rates of substitution in introns are nearly as high as those in flanking regions. Although these nucleotides do not encode amino acids, introns must be spliced out of the pre-mRNA for a functional protein to be produced, and particular sequences are required at the 5′ splice site, 3′ splice site, and branch point for correct splicing (see Chapter 14).



26.16 Different parts of genes evolve at different rates. The highest rates of nucleotide substitution are in sequences that have the least effect on protein function.

Description
The horizontal axis of the bar graph shows D N A and its parts as follows: 5 prime flanking region, 5 prime untranslated region, exon, intron, exon, 3 prime untranslated region, and 3 prime flanking region. The vertical-axis represents nucleotide substitution per site per year times 10 raised to the power of negative 9. The bar graph also shows a bar for pseudogene. Approximate data from the bar graph are as follows:

5 prime flanking region: 2 point 6.

5 prime untranslated region: 1 point 9.

Exon: It shows two bars at 0 point 9 and 4 point 7 labeled non-synonymous and synonymous, respectively. A caption pointing towards synonymous reads, “Nonsynonymous nucleotide substitutions alter the amino acid sequence, but synonymous ones do not.” A caption pointing towards non-synonymous reads, “Rates of nonsynonymous substitutions are lower in coding regions of exons.”

Intron: 3 point 8.

Exon: Blank.

3 prime untranslated region: 1 point 9.

3 prime flanking region: 4 point 5.

Pseudogene: 4 point 8; A caption pointing towards psuedogene bar reads, “but rates of substitution are much higher in nonfunctional D N A, such as pseudogenes.”

The D N A at the horizontal axis leads to pre-m R N A eliminating the flanking region on both sides. This further leads to m R N A and the intron at the center is also removed. Now, the m R N A has 5 prime untranslated region, two exons, and 3 prime untranslated region. This m R N A leads to protein.

Substitution rates are somewhat lower in the 5′ and 3′ untranslated regions of a gene. These regions are transcribed into RNA but do not encode amino acids. The 5′ untranslated region contains the ribosome-binding site, which is essential for translation, and the 3′ untranslated region contains sequences that may function in regulating mRNA stability and translation, so substitutions in these regions may have deleterious effects on organismal fitness and may not be tolerated.

The lowest rates of substitution are seen for nonsynonymous changes in exons, because these substitutions always alter the amino acid sequence of the protein and are often deleterious. High rates of substitution occur in *pseudogenes*, most of which are duplicate copies of genes that have been rendered nonfunctional by

mutations. Such genes no longer produce a functional product, so mutations in pseudogenes have little effect on the fitness of the organism.

In summary, there is a relation between the function of a DNA sequence and its rate of evolution: the highest rates of change are found where changes have the least effect on function. This observation fits with the neutral-mutation hypothesis, which predicts that molecular variation is not affected by natural selection. **› TRY** **PROBLEM 35**

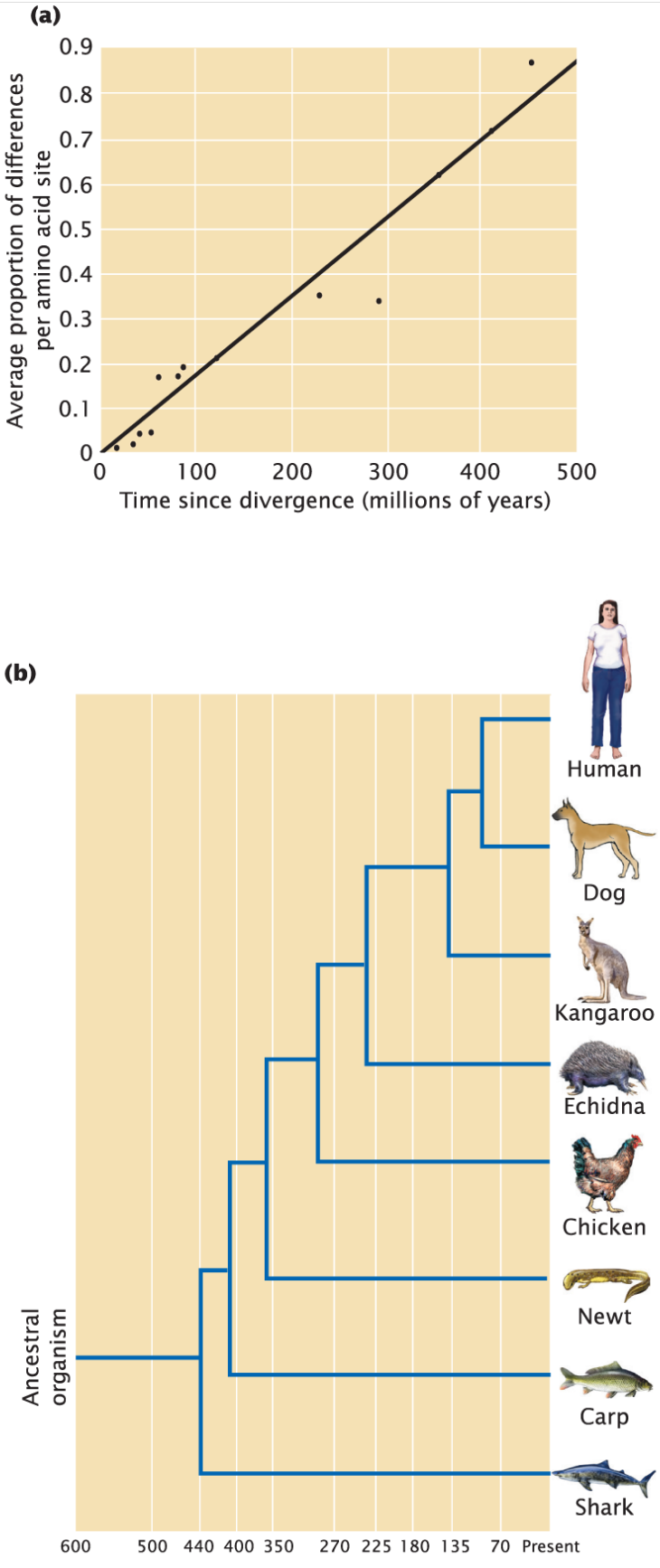
THINK-PAIR-SHARE Question 7

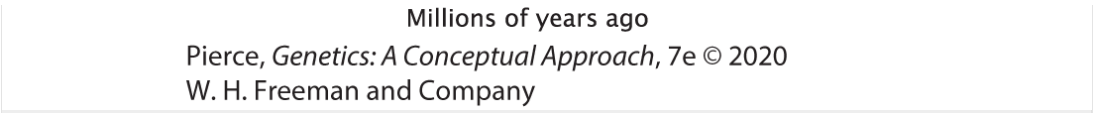
The Molecular Clock

The neutral-mutation hypothesis (neutral theory) proposes that evolutionary change at the molecular level takes place primarily through the fixation of neutral mutations by genetic drift. The rate at which one neutral mutation replaces another depends only on the mutation rate, which should be fairly constant for any particular gene. If the rate at which a protein evolves is roughly constant over time, the amount of molecular change that a protein has undergone can be used as a **molecular clock** to date evolutionary events.

For example, the enzyme cytochrome *c* could be examined in two organisms known from fossil evidence to have had a common ancestor 400 million years ago. By determining the number of differences between the cytochrome *c* amino acid sequences of the two organisms, we can calculate the number of substitutions that have occurred per amino acid site. Because we know when these organisms last shared a common ancestor, we can determine the rate at which substitutions are occurring. Knowing how fast the molecular clock ticks then allows us to use the number of molecular differences in cytochrome *c* to date other evolutionary events.

The molecular clock was proposed by Emile Zuckerkandl and Linus Pauling in 1965 as a possible means of dating evolutionary events on the basis of molecules in present-day organisms. A number of studies have examined the rate of evolutionary change in proteins (**Figure 26.17**) and in genes, and the molecular clock has been widely used to date evolutionary events when the fossil record is absent or ambiguous. For example, researchers used a molecular clock to estimate when Darwin's finches diverged from a common ancestor that originally colonized the Galápagos Islands. This clock was based on DNA sequence differences in the cytochrome *b* gene. The researchers concluded that the ancestor of Darwin's finches arrived in the Galápagos and began diverging some 2 million to 3 million years ago. The results of several studies have shown, however, that the molecular clock does not always tick at a constant rate, particularly over shorter time periods, and this method remains controversial.


(a)
Average proportion of differences per amino acid site
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
Time since divergence (millions of years)
(b)
Human
Dog
Kangaroo
Echidna
Chicken
Newt
Carp
Shark
Ancestral organism
600
500
440
400
350
270
225
180
135
70
Present



26.17 The molecular clock is based on the assumption of a constant rate of change in protein or DNA sequences. (a) Relation between the rate of amino acid substitution and time since divergence, based in part on the amino acid sequences of α-globin from the eight species shown in part *b*. The rate of evolution in protein and DNA sequences has been used as a molecular clock to date past evolutionary events. (b) Phylogeny of eight of the species that are plotted in part *a* and their approximate times of divergence based on the fossil record.

Description

Part “a” shows a scatter plot which depicts the relation between the rate of amino acid substitution and time since divergence. The horizontal axis represents time since divergence in millions of years ranging from 0 to 500 with intervals of 100. The vertical axis represents average proportion of differences per amino acid site ranging from 0 to 0 point 9 with intervals of 0 point 1. A straight line with positive slope starts from (0, 0) and ends at (500, 0 point 9). A few dots are present in two clusters near 0 and 0 point 2 of vertical axis. Two dots are below the middle of the line and one dot is above the end. Part “b” shows a phylogenetic tree with a scale at the bottom in millions of years ago from 600 to 70 and then present. The phylogenetic tree starts with ancestral organism dividing into two branches; shark at 440 m y a and the other branch further divides into carp at 400 m y a and newt at 360 m y a, leading to chicken at 290 m y a, which further leads to echidna at 240 m y a, which further branches to kangaroo at 135 m y a, then branches to dog at 100 m y a, and finally human at 100 m y a. All values are approximate.

CONCEPTS

Different genes and different parts of the same gene evolve at different rates. Those parts of genes that have the least effect on function tend to evolve at the highest rates. The idea that individual proteins and genes evolve at a constant rate and that the differences in the sequences of present-day organisms can be used to date past evolutionary events is referred to as the molecular clock.

✔ CONCEPT CHECK 6

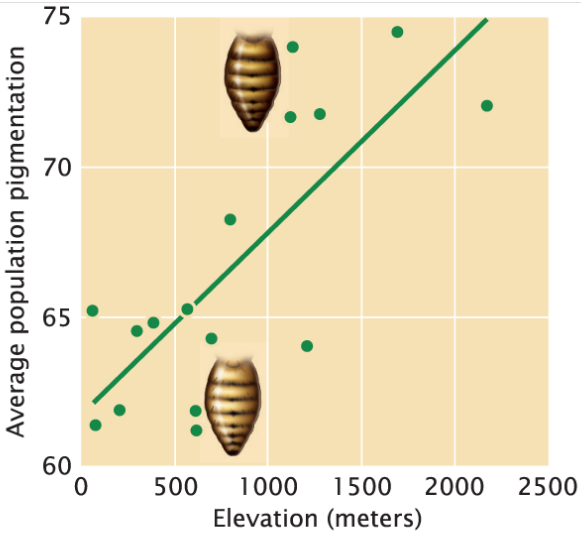
In general, which of the following is expected to exhibit the lowest rate of evolutionary change?

a. Synonymous changes in amino acid–coding regions of exons
b. Nonsynonymous changes in amino acid–coding regions of exons
c. Introns
d. Pseudogenes

Evolution Through Changes in Gene Regulation

One of the challenges of evolutionary biology is understanding the genetic basis of adaptation. Many evolutionary changes occur with relatively little genetic change. For example, humans and chimpanzees differ greatly in anatomy, physiology, and behavior, yet they differ at only about 4% of their DNA sequences (see the introduction to Chapter 17). Evolutionary biologists have long assumed that many anatomical differences result not from the evolution of new genes but rather from relatively small DNA differences that alter the expression of existing genes. Recent research in evolutionary genetics has focused on how evolution occurs through alteration of gene expression.

An example of adaptation that has occurred through changes in regulatory sequences is seen in the evolution of pigmentation in *Drosophila melanogaster* fruit flies in Africa. Most fruit flies are light tan in color, but flies in some African populations have much darker abdomens. These darker flies usually occur in mountainous regions at high elevations. Indeed, 59% of pigmentation variation among populations within Sub-Saharan Africa can be explained by differences in elevation (**Figure 26.18**). Researchers have demonstrated that these differences are genetically determined and that natural selection has favored darker pigmentation at high elevations. High-elevation populations are exposed to lower temperatures, and the darker pigmentation is assumed to help flies absorb more solar radiation and better regulate their body temperature in these environments.



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26.18 Sub-Saharan African populations of the fruit fly *Drosophila melanogaster* exhibit a positive association between pigmentation and elevation.

Description

The horizontal-axis represents elevation in meters ranging from 0 to 2,500 in increments of 500. The vertical axis represents average population pigmentation ranging from 60 to 75 in increments of 5. The best-fit line shows a steady positive slope that increases from (100, 63) to (2200, 75). Dots of moderate density are shown near the line. The dots are somewhat denser from (100, 62) to (700, 65). Two pictures of fruit fly are shown; one below and one above the line between 500 and 100 meter elevation.

How did flies at high elevations evolve darker color? Genetic studies indicate that the dark abdominal pigmentation seen in flies from these populations results from variation at or near a locus called *ebony*. The *ebony* locus encodes a multifunctional enzyme that produces a yellow exoskeleton; the absence of this enzyme produces a dark phenotype. Sequencing of the *ebony* locus of flies from light and dark populations found no differences in the coding region of the *ebony* gene. However, molecular analysis revealed a marked reduction in the amount of *ebony* mRNA in darker flies, suggesting that the difference in pigmentation is due not to mutations in the *ebony* gene itself but rather to changes in its expression. Further investigation detected genetic differences within an enhancer that is about 3600 bp upstream of the *ebony* gene. Dark and light flies differed in over 120

nucleotides scattered over 2400 bp of the enhancer. By experimentally creating enhancers with different combinations of these mutations, researchers determined that five of the mutations are responsible for the majority of the difference in pigmentation.

The results of these studies suggest that over time, high-elevation populations accumulated multiple mutations in the enhancer, which reduced the expression of the *ebony* locus and caused darker pigmentation. Further analysis suggested that these mutations were added sequentially. Some of the mutations are widespread throughout Africa; it is assumed that these existing mutations were favored by natural selection in high-elevation populations and increased in frequency because they helped the flies thermoregulate in colder environments. Other mutations that are seen only in the high-elevation populations probably arose as new mutations within those populations and were quickly favored by natural selection.

THINK-PAIR-SHARE Question 8

Genome Evolution

The vast store of sequence data now available in DNA databases has been a source of insight into evolutionary processes. Whole-genome sequences are also providing new information about how genomes evolve and the processes that shape the size, complexity, and organization of genomes.

EXON SHUFFLING

Many proteins are composed of groups of amino acids, called domains, that have discrete functions or contribute to the molecular structure of a protein. For example, in Section 16.1, we considered the DNA-binding domains of proteins that

regulate gene expression. Analyses of gene sequences from eukaryotic organisms indicate that exons often encode discrete functional domains of proteins.

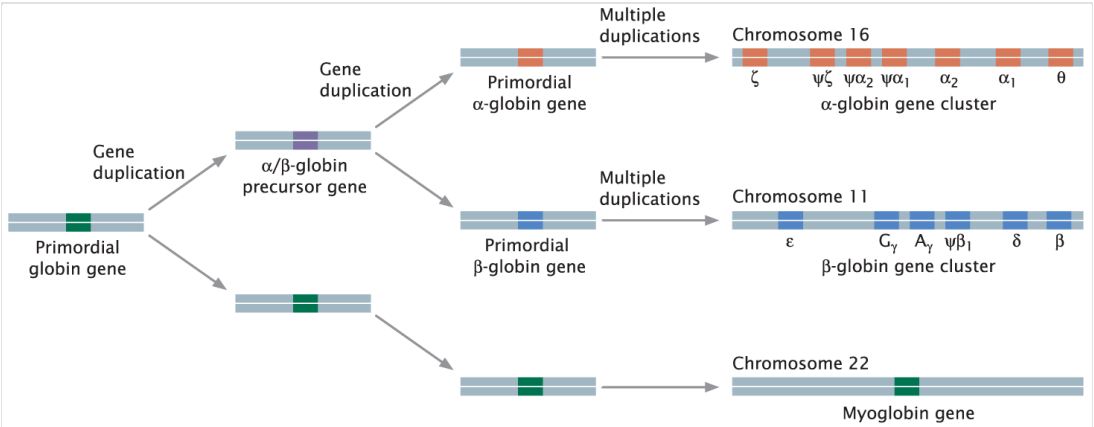
Some genes elongated and evolved new functions when one or more exons duplicated and underwent divergence. For example, the human serum-albumin gene is made up of three copies of a sequence that encodes a protein domain consisting of 195 amino acids. Additionally, the genes that encode human immunoglobulins have undergone repeated tandem duplications, creating many similar *V*, *J*, *D*, and *C* segments (see Section 22.6) that enable the immune system to respond to almost any foreign substance that enters the body.

A comparison of DNA sequences from different genes reveals that new genes have repeatedly evolved through a process called **exon shuffling**, in which exons of different genes are exchanged, creating genes that are mosaics of other genes. For example, tissue plasminogen activator (TPA) is an enzyme that contains four domains of three different types, called kringle, growth factor, and finger. Each domain is encoded by a different exon. The gene for TPA is believed to have acquired its exons from other genes that encode different proteins: the kringle exon came from the plasminogen gene, the growth-factor exon came from the epidermal growth factor gene, and the finger exon came from the fibronectin gene. The mechanism by which exon shuffling takes place is poorly understood, but new proteins with different combinations of functions encoded by other genes have repeatedly evolved by this mechanism.

GENE DUPLICATION

New genes have also evolved through the duplication of whole genes and their subsequent divergence. This process creates **multigene families**, sets of genes that are similar in sequence but encode different products. For example, humans possess 13 different genes found on chromosomes 11 and 16 that encode globin-like molecules, which take part in oxygen transport (**Figure 26.19**). All of these genes have a similar structure, with three exons separated by two introns. They

are assumed to have evolved through repeated duplication and divergence from a single globin gene in a distant ancestor. This ancestral gene is thought to have been most similar to the present-day myoglobin gene, and it was probably first duplicated to produce an α/β-globin precursor gene and the myoglobin gene. The α/β-globin gene then underwent another duplication to give rise to a primordial α-globin gene and a primordial β-globin gene. Subsequent duplications led to multiple α-globin and β-globin genes. Similarly, vertebrates contain four clusters of *Hox* genes, each comprising from 9 to 11 genes. *Hox* genes play an important role in development (see pp. 684–685 in Chapter 22).



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26.19 The human globin gene family has evolved through successive gene duplications. A primordial globin gene duplicated into an α/β-globin precursor gene and a gene for myoglobin. Later, the α/β-globin precursor gene duplicated, giving rise to α-globin and β-globin genes, which then underwent further duplications. ψ designates pseudogenes.

Description

The illustration starts with a double stranded linear D N A having primordial globin gene. It branches two parts; one gene remains the same, while the other undergoes gene duplication. The similar gene leads to another similar gene and finally leads to chromosome 22 with myoglobin gene. The gene which undergoes gene duplication shows an alpha/beta-globin precursor gene. It again bifurcates and gene duplication leads to one chromosome with primordial alpha-globin gene and one with primordial beta-globin gene. Multiple duplications occur on both chromosomes. Primordial alpha-globin gene leads to chromosome 16 showing alpha-globin gene clusters as follows: zeta, psi-zeta, psi-alpha 2, psi-alpha 1, alpha 2, alpha 1, and theta. Primordial beta-globin gene leads to chromosome 11 showing beta-globin gene cluster as follows: epsilon, G subscript gamma, A subscript gamma, psi-beta 1, lowercase delta, and beta.

Some gene families include genes that are arrayed in tandem on the same chromosome; others are dispersed among different chromosomes. Gene duplication is a common occurrence in eukaryotic genomes; for example, about 5% of the human genome consists of duplicated segments.

Gene duplication provides a mechanism for the generation of new genes with novel functions. Once a gene has been duplicated, there are two copies of that gene, one of which is then free to change and potentially take on a new function. The extra copy of the gene may, for example, become active at a different time in development, or be expressed in a different tissue, or even diverge and encode a protein containing different amino acids. The most common outcome of gene duplication, however, is that one copy acquires a mutation that renders it nonfunctional and becomes a pseudogene. Pseudogenes are common in the genomes of complex eukaryotes; the human genome is estimated to contain as many as 20,000 pseudogenes.

WHOLE-GENOME DUPLICATION

In addition to the duplication of individual genes, whole genomes of some organisms have been duplicated in the past. For example, a comparison of the genome of the yeast *Saccharomyces cerevisiae* with the genomes of other fungi reveals that *S. cerevisiae*, or one of its immediate ancestors, underwent a whole-genome duplication that generated two copies of every gene. Many of the copies subsequently acquired new functions; others acquired mutations that destroyed their original function and then diverged into random DNA sequences.

Whole-genome duplication can take place through polyploidy, as we saw in Section 26.3. During their evolution, plants have undergone a number of whole-genome duplications in this way. While polyploidy is less frequent in animals, genetic evidence suggests that several whole-genome duplication events have occurred during animal evolution. In 1970, Susumu Ohno proposed that early vertebrates underwent two rounds of genome duplication. Called the 2R

hypothesis, this idea has been controversial, but recent data from genome sequencing have provided support for it. Among vertebrates, polyploidy has been particularly common in amphibians.

HORIZONTAL GENE TRANSFER

Traditionally, scientists assumed that organisms acquire their genomes through vertical transmission—transfer through the reproduction of genetic information from parents to offspring—and most phylogenetic trees assume vertical transmission of genetic information. Findings from DNA sequence studies reveal that DNA sequences are sometimes transmitted by horizontal gene transfer, in which DNA is transferred between individuals of different species (see Section 9.3). This process is especially common among bacteria, and there are a number of documented cases in which genes have been transferred from bacteria to eukaryotes. The extent of horizontal gene transfer among eukaryotic organisms is controversial, as there are few well-documented cases. Horizontal gene transfer can obscure phylogenetic relationships and make the reconstruction of phylogenetic trees difficult.

One apparent case of horizontal gene transfer among eukaryotes is the presence in some aphids of genes for enzymes that synthesize carotenoids. Carotenoids are colored compounds produced by bacteria, archaea, fungi, and plants. Many animals also possess carotenoids, but they lack the enzymes necessary to make the compounds themselves; in almost all cases, animals obtain carotenoids from their food.

Aphids—small insects that feed on plants—possess carotenoids, which are responsible for color differences between and within species. Some aphids are green and contain α-, β-, and γ-carotene, which are all yellow carotenoids. Other aphids are red or brown and contain lycopene or torulene, carotenoids that are red. One species, the pea aphid (*Acyrthosiphon pisum*), contains both green and red individuals, and these color differences are genetically inherited. Many

researchers previously assumed that the color differences were due to carotenoids that were acquired in the aphids' food.

The sequencing of the entire genome of *A. pisum* provided researchers with the opportunity to determine whether the aphids possess their own genes for carotenoid synthesis. Examination of the genome sequence revealed that pea aphids have several genes that code for carotenoid-synthesizing enzymes. Interestingly, these genes are closely related to carotenoid-synthesizing genes found in some fungi. The evidence suggests that in the distant past, an aphid acquired its carotenoid genes from a fungus through horizontal gene transfer and then passed the genes to other aphids through vertical transmission.

CONCEPTS

New genes may evolve through the duplication of exons, the shuffling of exons, the duplication of genes, and the duplication of whole genomes. Genes can be passed among different species through horizontal gene transfer.

Chapter 26 Review

CHAPTER SUMMARY

Section 26.1 Learning Objective: Be able to explain what biological evolution is and how it occurs.

- Evolution is genetic change that takes place within a group of organisms. It is a two-step process: (1) genetic variation arises, and (2) genetic variants change in frequency.
- Anagenesis refers to change within a single lineage; cladogenesis is the splitting of one lineage into two.

Section 26.2 Learning Objective: Be able to discuss possible explanations for the observation that many populations contain high levels of genetic variation.

- Molecular methods offer a number of advantages for the study of evolution.
- Most natural populations contain large amounts of genetic variation. The neutral-mutation hypothesis proposes that most molecular variants are selectively neutral and that their evolution is shaped largely by mutation and genetic drift. Some molecular variation is maintained by balancing selection.

Section 26.3 Learning Objective: Be able to explain how new species arise through the evolution of reproductive isolating mechanisms.

- A species can be defined as a group of organisms that are capable of interbreeding with one another and are reproductively isolated from the members of other species.
- Species are prevented from exchanging genes by prezygotic or postzygotic reproductive isolating mechanisms.
- Allopatric speciation arises when a geographic barrier prevents gene flow between two populations. With the passage of time, the two populations acquire genetic differences that may lead to reproductive isolation.
- Sympatric speciation arises when reproductive isolation evolves in the absence of any geographic barrier. It may arise under special circumstances.
- Some species arise only after populations have undergone considerable genetic differentiation; others arise after changes have taken place in only a few genes.

Section 26.4 Learning Objective: Be able to explain how phylogenetic trees are used to study evolutionary relationships, how to interpret phylogenetic trees, and how they are constructed.

- A phylogeny can be represented by a phylogenetic tree, consisting of nodes that represent organisms and branches that represent their evolutionary connections.
- Approaches to constructing phylogenetic trees include the distance approach, the maximum parsimony approach, and the

maximum likelihood and Bayesian methods.

Section 26.5 Learning Objective: Be able to describe patterns of molecular evolution.

- Different parts of a gene show different amounts of genetic variation. In general, those parts that have the least effect on function evolve at the highest rates.
- The molecular-clock hypothesis proposes a constant rate of molecular change, providing a means of dating evolutionary events by looking at sequence differences between present-day organisms.
- Genome evolution takes place through the duplication and shuffling of exons, the duplication of genes to form multigene families, whole-genome duplication, and horizontal transfer of genes.

IMPORTANT TERMS

evolution
anagenesis
cladogenesis
neutral-mutation hypothesis
balancing selection
species
biological species concept
reproductive isolating mechanism
prezygotic reproductive isolating mechanism

ecological isolation
behavioral isolation
temporal isolation
mechanical isolation
gametic isolation
postzygotic reproductive isolating mechanism
hybrid inviability
hybrid sterility
hybrid breakdown
speciation
allopatric speciation
sympatric speciation
phylogeny
phylogenetic tree
branch
node
rooted tree
gene tree
molecular clock
exon shuffling
multigene family

ANSWERS TO CONCEPT CHECKS

1. First, genetic variation arises. Then, various evolutionary forces cause changes in the frequency of genetic variants.
2. c

3. b
4. Genetic drift can bring about changes in the allelic frequencies of a population and lead to genetic differences among populations. Genetic differentiation is the cause of postzygotic and prezygotic reproductive isolation between populations that leads to speciation.
5. c
6. b

Chapter 26 Assessment

COMPREHENSION QUESTIONS

Section 26.1

1. How is biological evolution defined?
2. What are the two steps in the process of evolution?
3. How is anagenesis different from cladogenesis?

Section 26.2

4. What are some of the advantages of using molecular data in evolutionary studies?
5. What is the key difference between the neutral-mutation hypothesis and balancing selection?
6. Describe some of the methods that have been used to study variation in DNA.

Section 26.3

7. What is the biological species concept?

8. What is the difference between prezygotic and postzygotic reproductive isolating mechanisms? List some different types of each.

9. What is the basic difference between allopatric and sympatric modes of speciation?

10. Briefly outline the process of allopatric speciation.

11. What are some of the difficulties with sympatric speciation?

12. Briefly explain how switching from hawthorn fruits to apples has led to genetic differentiation and partial reproductive isolation in *Rhagoletis pomonella*.

Section 26.4

13. Draw a simple phylogenetic tree, and identify a node, a branch, and an outgroup.

14. Briefly describe differences among the distance approach, the maximum parsimony approach, and the maximum likelihood approach to the reconstruction of phylogenetic trees.

Section 26.5

15. Outline the different rates of evolution that are typically seen in different parts of a protein-encoding gene. What might account for these differences?

16. What is the molecular clock?

17. What is exon shuffling? How can it lead to the evolution of new genes?

18. What is a multigene family? What processes produce multigene families?

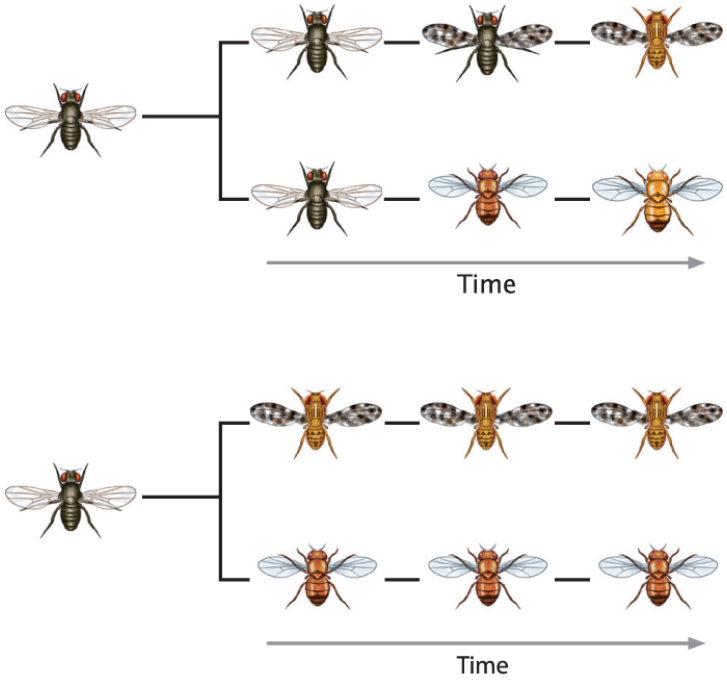
19. Define *horizontal gene transfer*. What problems does it cause for evolutionary biologists?

APPLICATION QUESTIONS AND PROBLEMS

Section 26.1

20. CONNECT Are biological evolution and natural selection the same? Why or why not? (See Section 25.4 for a discussion of evolutionary forces.)

21. *The following illustrations represent two different patterns of evolution. Briefly discuss the differences in these two patterns with regard to how evolutionary change occurs with respect to time and splitting of lineages.



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Description

The first part shows a black fly with spotless wings leading to two branches. Each branch has three flies. The first branch shows a black fly with spotless wings, a black fly with spotted wings, and a yellow fly with spotted wings. The second branch shows a black fly with spotless wings, an orange fly with spotless wings, and a yellow fly with spotless wings.

The second part shows a black fly with spotless wings leading to two branches. Each branch has three flies. The first branch shows three yellow flies with spotted wings. The second branch shows three orange flies with spotless wings.

22. CONNECT Researchers studying horn size among bighorn sheep on Ram Mountain found that the narrow-sense heritability of horn size in males was 0.4. The average horn length among all males in 1980 was about 65 cm. If the average horn length of selected males in that year (those that

reproduced) was 50 cm, what would you predict would be the horn size of the offspring of the males that reproduced? See Chapter 24 for a discussion of the response to selection.

Section 26.2

23. CONNECT The neutral-mutation hypothesis proposes that most of the molecular variation observed in nature is selectively neutral and its evolution is largely shaped by mutation and genetic drift (see Chapter 25). What is genetic drift, and what are the effects of genetic drift on populations?

Section 26.3

24. CONNECT Researchers have discovered a strong association between different beak shapes in Darwin's finches and a gene (*ALX1*) that encodes a transcription factor called Aristaless-like homeobox 1. How might genetic changes in a gene for a transcription factor affect a phenotypic trait like beak shape in birds? (See Chapter 17.)

25. *Which of the isolating mechanisms listed in **Table 26.1** have partly evolved between apple and hawthorn host races of *Rhagoletis pomonella*, the apple maggot fly?

26. DATA ANALYSIS We considered the sympatric evolution of reproductive isolating mechanisms in host races of *Rhagoletis pomonella*, the apple maggot fly. The wasp *Diachasma alloeum* parasitizes apple maggot flies, laying its eggs on the larvae of the flies. Immature wasps hatch from the eggs and feed on the fly larvae. Research by Andrew Forbes and his colleagues (Forbes et al. 2009. *Science* 323:776–779) demonstrated that wasps that parasitize the apple race of *R. pomonella* are genetically differentiated from those that parasitize the hawthorn race. They also found that wasps that prey on the apple race of the flies are attracted to odors from apples, whereas wasps that prey on the hawthorn race are attracted to odors from hawthorn fruits. Propose an explanation for how genetic differences might have evolved between the wasps that parasitize the two host races of *R. pomonella*. How might these differences lead to speciation in the wasps?

27. CONNECT New species that arise through polyploidy are usually allopolyploids. Why doesn't autopolyploidy usually lead to new species? See Chapter 8 for a discussion of autopolyploidy and allopolyploidy.

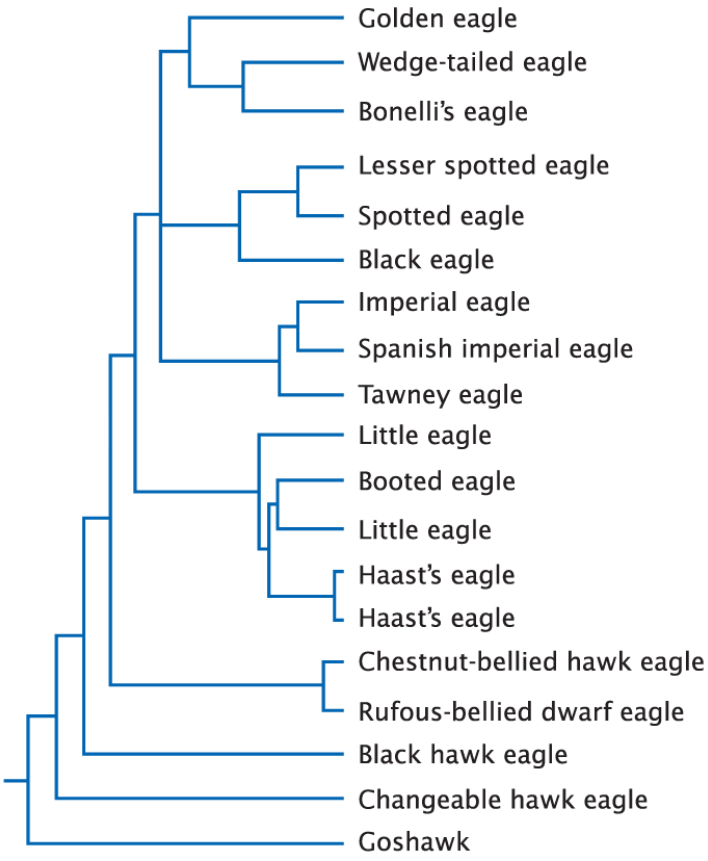
28. Which of the salt-marsh grasses in **Figure 26.10** is a polyploid?
    a. *Spartina maritima*
    b. *Spartina alterniflora*
    c. Hybrid between *Spartina maritima* and *Spartina alterniflora*
    d. *Spartina anglica*
    e. Both c and d

Section 26.4

29. How many rooted trees are theoretically possible for a group of seven organisms? How many for twelve organisms?
30. Align the sequences below so as to maximize their similarity. What is the minimum number of evolutionary steps that separate these two sequences?

TTGCAAAC
TGAAACTG

31. DATA ANALYSIS *Michael Bunce and his colleagues in England, Canada, and the United States extracted and sequenced mitochondrial DNA from fossils of Haast's eagle, a gigantic eagle that was driven to extinction 700 years ago when humans first arrived in New Zealand (M. Bunce et al. 2005. *PLOS Biology* 3:44–46). Using mitochondrial DNA sequences from living eagle species and those from Haast's eagle fossils, they created the accompanying phylogenetic tree. On the basis of this tree, determine the following: (a) which living eagle species are most closely related to Haast's eagle? (b) which living eagle species is most distantly related to Haast's eagle? (c) which species is more closely related to Haast's eagle, the Chestnut-bellied hawk eagle or the Golden eagle?



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[Data from M. Bunce et al. 2005. *PLOS Biology* 3:44–46.]

Description

The phylogentic tree starts from Goshawk at the bottom, which leads to Changeable hawk eagle, which further leads to black hawk eagle. The black hawk eagle leads to chestnut-bellied hawk eagle and rufous-bellied dwarf eagle. It further leads to two branches. One has haast's eagle and haast's eagle leading to little eagle and booted eagle leading to little eagle. The other is further divided into three branches. One has Tawney eagle leading to Spanish imperial eagle and imperial eagle. The second has black eagle leading to spotted eagle and lesser spotted eagle. The third has Bonelli's eagle and wedge-tailed eagle leading to golden eagle. The closest node to the right of haast's eagle in the phylogenetic tree are little eagle and booted eagle. The node farthest to the left of haast's eagle is Goshawk and that to the right is golden eagle.

32. On the basis of the phylogeny of Darwin's finches shown in **Figure 26.7**, predict which two species in each of the following groups will be the most similar genetically.
    a. *Camarhynchus parvulus*, *Camarhynchus psittacula*, *Camarhynchus pallidus*
    b. *Camarhynchus parvulus*, *Camarhynchus pallidus*, *Platyspiza crassirostris*
    c. *Geospiza difficilis*, *Geospiza conirostris*, *Geospiza scandens*
    d. *Camarhynchus parvulus*, *Certhidea fusca*, *Pinaroloxias inornata*

Section 26.5

33. If a gene had similar rates of nonsynonymous and synonymous substitutions, what might that suggest about the evolution of that gene?

34. CONNECT Rates of nucleotide change are usually higher among synonymous codons than among nonsynonymous codons (see **Figure 26.16**). Why do synonymous codons (see Chapter 15) frequently differ only at the third position of the codon?

35. *Based on the information provided in **Figure 26.16**, do introns or 3′ untranslated regions of a gene have higher rates of nucleotide substitution? Explain why.

36. CONNECT **Figure 26.16** shows that a gene's 3′ untranslated region (3′ UTR) exhibits lower rates of nucleotide substitution than do introns. What sequences in the 3′ untranslated region of a gene have important functions that might limit substitutions in this region? (See Chapter 14.)

37. CONNECT Horizontal gene transfer is common in bacteria. What processes in bacteria result in horizontal gene transfer? (See Chapter 9.)

CHALLENGE QUESTIONS

Section 26.3

38. Explain why natural selection may cause prezygotic reproductive isolating mechanisms to evolve if postzygotic reproductive isolating mechanisms are already present, but can never cause the evolution of postzygotic reproductive isolating mechanisms.

39. Polyploidy is very common in flowering plants: approximately 40% of all flowering plant species are polyploids. Although polyploidy exists in many different animal groups, it is much less common. Why is polyploidy more common in plants than in animals? Give one or more possible reasons.

ACTIVE LEARNING: THINK-PAIR-SHARE QUESTIONS

Section 26.1

1. Consider Theodosius Dobzhansky's remark, "Nothing in biology makes sense except in the light of evolution." Why is evolution so important to the field of biology? Give some specific examples of how the theory of evolution helps us make sense of biology.

2. Evolution is often misunderstood and misinterpreted. What are some common misconceptions about evolution? How are these misconceptions wrong?

Section 26.3

3. Examine **Table 26.1**, which lists different types of reproductive isolating mechanisms. Try to come up with at least one example of an organism that is reproductively isolated from other species by each of the mechanisms listed in the table. Are some groups of organisms more likely to exhibit one type of mechanism than another? Give some examples.

4. The biological species concept is based on the assumption that species are reproductively isolated and do not share genes. And yet a number of organisms that are considered different species

hybridize (mate and exchange genes). Hybridization between different species is more common in plants than in animals. Propose some possible reasons for this difference.

5. One of Darwin's finches, the medium ground finch (*Geospiza fortis*), is found on the small island of Daphne Major. These finches are seed-eating birds. A major drought occurred on the island in 1977. Following the drought, the average beak size of medium ground finches had increased about 3%–4%. Why might a drought lead to an evolutionary change in beak size? Propose a hypothesis, and explain how you could go about testing it.

Section 26.5

6. In most cases, the rate of synonymous substitution for a gene is higher than the rate of nonsynonymous substitution. Sometimes, however, the rate of nonsynonymous substitution is higher. When would you expect to see this? What might bring it about?

7. Do the data in **Figure 26.16** support the predictions of the neutral-mutation hypothesis, or do they suggest that balancing selection is occurring? Explain your reasoning.

8. What changes, if any, would you predict in the pigmentation of *Drosophila melanogaster* with increased global warming? What type(s) of genetic changes would you expect to see? Be as specific as you can.

ACTIVE LEARNING: CONCEPT MAPPING EXERCISES

Section 26.2

1. Create a concept map that shows the relationships between the following concepts: genetic variation, phenotypic variation, molecular variation, selectively neutral variation, balancing selection, neutral-mutation hypothesis, fitness differences.

Section 26.3

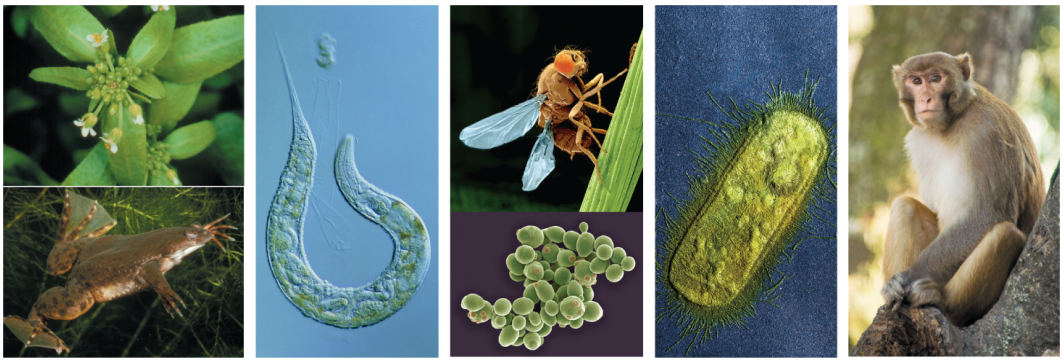
2. Draw a concept map that summarizes the biological species concept by including the following terms: species, biological species concept, hybrid inviability, postzygotic reproductive isolating mechanisms, temporal isolation, reproductive isolating mechanisms, hybrid sterility, prezygotic reproductive isolating mechanisms, ecological isolation, gametic isolation, behavioral isolation, hybrid breakdown, mechanical isolation.

Section 26.4

3. Draw a concept map that summarizes phylogenies by including at least the following terms: phylogeny, evolutionary relationships, phylogenetic tree, nodes, branches, present-day organisms, outgroup, distance approach, maximum likelihood, Bayesian methods, maximum parsimony approach.

SaplingPlus Self-study tools that will help you practice what you've learned and reinforce this chapter's concepts are available online. Go to www.saplinglearning.com.

Reference Guide to Model Genetic Organisms



Left to right: Dr. Jeremy Burgess/Science Source; © Leszczynski, Zigmund/Animals Animals; Sinclair Stammers/Science Source; Eye of Science/Science Source; Steve Gschmeissner/Science Photo Library/Alamy; Biophoto Associates/Science Source; © Gelia, Dreamstime.com.

Model genetic organisms possess characteristics that make them useful for genetic studies. Shown are several organisms commonly used in genetic studies.

What did Lou Gehrig, the finest first baseman in major league history, and Stephen Hawking, one of the world's most famous theoretical physicists, have in common? They both suffered from amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease), a degenerative neurological disease that leads to progressive weakness and wasting of skeletal muscles.

Most cases of ALS are sporadic, appearing in people who have no family history of the disease, such as in Lou Gehrig, discussed in the introduction to Chapter 18. However, about 10% of the cases run in families and are inherited. In 2004, geneticists discovered a

large Brazilian family with multiple cases of ALS. Genetic analysis revealed that ALS in this family is due to a mutation in a gene called *VABP*, which encodes a vesicle-associated membrane protein. This gene is distinct from the *C9orf72* gene that causes ALS in other families (see Chapter 18), illustrating the principle that genetic diseases can be caused by mutations in several different genes.

To better understand how mutations in *VABP* lead to the symptoms of ALS, geneticists turned to an unlikely subject—the fruit fly *Drosophila melanogaster*. Fruit flies don't have ALS, but they do possess a gene very similar to *VABP*. Using a wide array of techniques that have been developed for genetically manipulating fruit flies, geneticists created transgenic flies with the mutant sequence of the *VABP* gene that causes ALS in humans. These flies are a disease model for ALS and are being used to better understand what the gene *VABP* does normally and how its disruption can lead to ALS.

The field of genetics has been greatly influenced and shaped by a few key organisms—called model genetic organisms—whose characteristics make them particularly amenable to genetic studies. The use of *Drosophila* for studying ALS in humans illustrates the power of this approach. Because features of genetic systems are common to many organisms, research conducted on one species can often be a source of insight into the genetic systems of other species. This commonality of genetic function means that

geneticists can focus their efforts on model organisms that are easy to work with and likely to yield results.

Model genetic organisms possess life cycles and genomic features that make them well-suited to genetic study and analysis. Some key features possessed by many model genetic organisms include

- a short generation time, so that several generations of genetic crosses can be examined in reasonable time;
- the production of numerous progeny, which allows genetic ratios to be easily observed;
- the ability to carry out and control genetic crosses in the organism;
- the ability to be reared in a laboratory environment, requiring little space and few resources to maintain;
- the availability of numerous genetic variants; and
- an accumulated body of knowledge about their genetic systems.

In recent years, the genomes of many model genetic organisms have been completely sequenced, greatly facilitating their use in genetic research.

Not all model organisms possess all these characteristics. However, each model genetic organism has one or more features that make it useful for genetic analysis. For example, corn cannot be easily grown in the laboratory (and usually isn't) and it has a relatively

long generation time, but it produces numerous progeny, and there are many genetic variants of corn available for study.

This reference guide highlights six model genetic organisms with important roles in the development of genetics: the fruit fly (*Drosophila melanogaster*), the bacterium (*Escherichia coli*), the roundworm (*Caenorhabditis elegans*), the thale-cress plant (*Arabidopsis thaliana*), the house mouse (*Mus musculus*), and yeast (*Saccharomyces cerevisiae*). These six organisms have been widely used in genetic research and instruction. A number of other organisms are also used as model systems in genetics, including corn (*Zea mays*), zebrafish (*Danio rerio*), clawed frog (*Xenopus laevis*), bread mold (*Neurospora crassa*), rat (*Rattus norvegicus*), and Rhesus macaque (*Macaca mulatta*), just to mention a few.

The Fruit Fly *Drosophila melanogaster*

Drosophila melanogaster, a fruit fly, was among the first organisms used for genetic analysis, and today it is one of the most widely used and best known genetically of all organisms. It has played an important role in studies of linkage, epistasis, chromosome genetics, development, behavior, and evolution. Because all organisms use a common genetic system, understanding a process such as replication or transcription in fruit flies helps us to understand these same processes in humans and other eukaryotes.

Drosophila is a genus of more than 1000 described species of small flies (about 2 to 3 mm in length) that frequently feed and reproduce on fruit, although they rarely cause damage and are not considered economic pests. The best known and most widely studied of the fruit flies is *D. melanogaster*, but genetic studies have also been extended to many other species of the genus. *D. melanogaster* first began to appear in biological laboratories about 1900. After first taking up breeding experiments with mice and rats, Thomas Hunt Morgan began using fruit flies in experimental studies of heredity at Columbia University. Morgan's laboratory, located on the top floor of Schermerhorn Hall, became known as the Fly Room (see Figure 4.11b). To say that the Fly Room was unimpressive is an understatement. The cramped room, only about 16 by 23 feet, was filled with eight desks, each occupied by a student and his experiments. The primitive laboratory equipment consisted of little

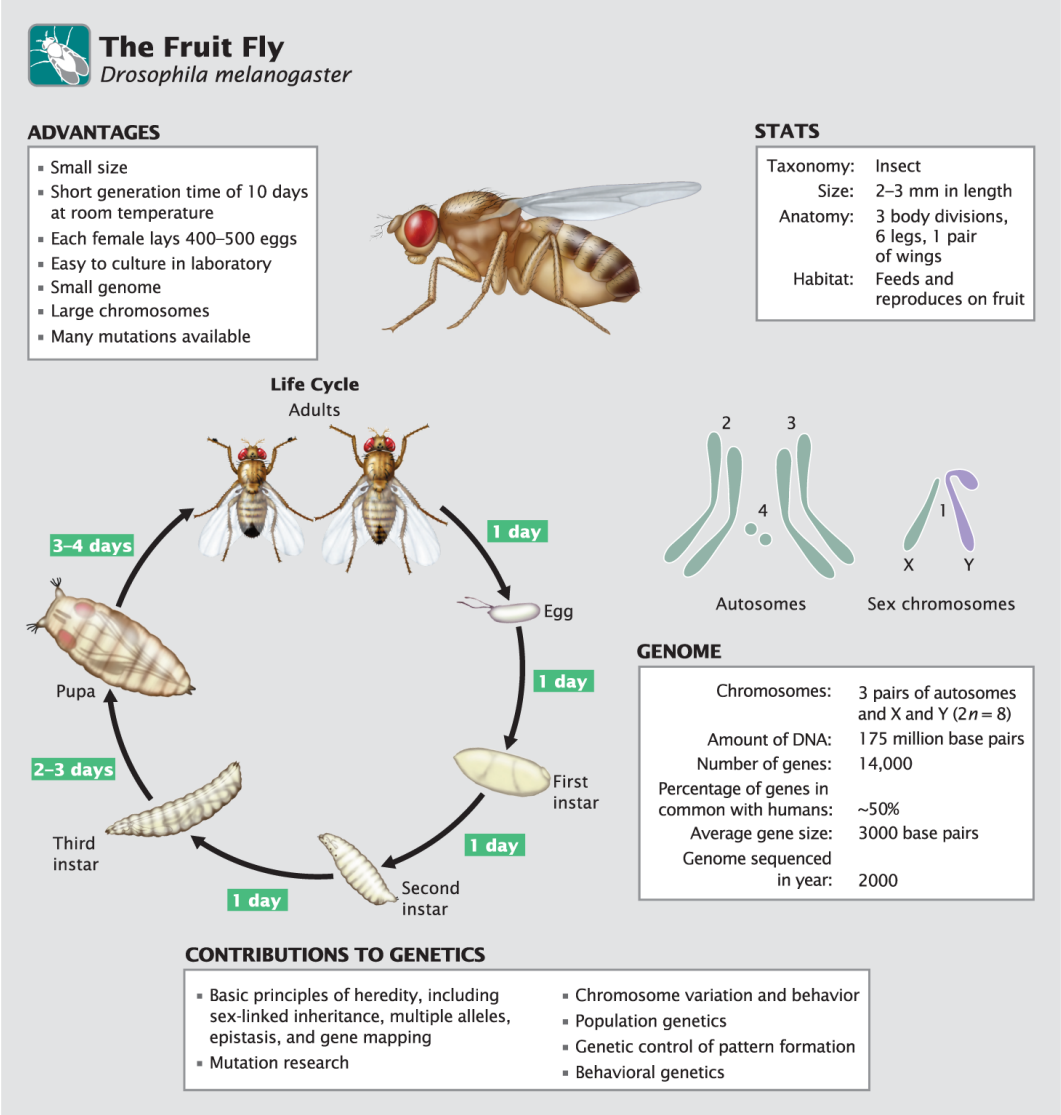
more than milk bottles for rearing the flies and hand-held lenses for observing their traits. Later, microscopes replaced the hand-held lenses, and crude incubators were added to maintain the fly cultures, but even these additions did little to increase the physical sophistication of the laboratory. Morgan and his students were not tidy: cockroaches were abundant (living off spilled *Drosophila* food), dirty milk bottles filled the sink, ripe bananas—food for the flies—hung from the ceiling, and escaped fruit flies hovered everywhere. Despite its physical limitations, the Fly Room was the source of some of the most important research in the history of biology. There was daily excitement among the students, some of whom initially came to the laboratory as undergraduates. The close quarters facilitated informality and the free flow of ideas. Morgan and the Fly Room illustrate the tremendous importance of "atmosphere" in producing good science. Morgan and his students eventually used *Drosophila* to elucidate many basic principles of heredity, including sex-linked inheritance, epistasis, multiple alleles, and gene mapping.

ADVANTAGES OF *D. MELANOGASTER* AS A MODEL GENETIC ORGANISM

Drosophila's widespread use in genetic studies is no accident. The fruit fly has a number of characteristics that make it an ideal subject for genetic investigations. Compared with other organisms, it has a relatively short generation time; fruit flies will complete an entire

generation in about 10 days at room temperature, so several generations can be studied within a few weeks. Although *D. melanogaster* has a short generation time, it possesses a complex life cycle, passing through several different developmental stages, including egg, larva, pupa, and adult. A female fruit fly is capable of mating within 8 hours of emergence and typically begins to lay eggs after about 2 days. Fruit flies also produce a large number of offspring, laying as many as 400 to 500 eggs in a 10-day period. Thus, large numbers of progeny can be obtained from a single genetic cross.

Another advantage is that fruit flies are easy to culture in the laboratory. They are usually raised in small glass vials or bottles and are fed easily prepared, pastelike food consisting of bananas or corn meal and molasses. Males and females are readily distinguished, and virgin females are easily isolated, facilitating genetic crosses. The flies are small, requiring little space—several hundred can be raised in a half-pint bottle—but they are large enough for many mutations to be easily observed with a hand lens or a dissecting microscope.



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Description

The illustration shows a Drosophila. The advantages are as follows: Small size, short generation time of 10 days at room temperature, each female lays 400 to 500 eggs, small genome, easy to culture in laboratory, large chromosomes, and many mutations available. Stats are as follows: Taxonomy: Insect; size: 2 to 3 m m in length; anatomy: 3 body divisions, 6 legs, 1 pair of wings; and habitat: feeds and reproduces on fruit. The illustration then shows autosomes 2, 3, and 4. Chromosomes 2 and 3 are long and V-shaped. The two chromosomes labeled 4 are spherical. All the chromosomes are in pair. Sex chromosomes shows X and Y chromosomes. The X-chromosome is long and has one arm only. The Y-chromosome has shorter upper arm than its lower arm. Genome shows

the following characteristics: Chromosomes: 3 pairs of autosomes and X and Y (2 n equals 8); amount of D N A: 175 million base pairs; number of genes: 14,000; percentage of genes in common with humans: approximately 50 percent; average gene size: 3000 base pair; and genome sequenced in year: 2000. Contributions to genetics are as follows: Basic principles of heredity, including sex-linked inheritance, multiple alleles, epistasis, and gene mapping; mutation research; chromosome variation and behavior; population genetics; genetic control of pattern formation; and behavioral genetics. Life cycle is as follows: Two adult drosophilas are shown. After 1 day, they produce an egg. The egg grows into first instar in one day, then the second instar in one day, and the third instar in one day. The third instar grows into pupa in 2 to 3 days. Pupae grow into adults in 3 to 4 days.

Finally, *D. melanogaster* is the organism of choice for many geneticists because it has a relatively small genome consisting of 175 million base pairs of DNA, which is only about 5% the size of the human genome. It has four pairs of chromosomes: three pairs of autosomes and one pair of sex chromosomes. The X chromosome (designated chromosome 1) is large and acrocentric, whereas the Y chromosome is large and submetacentric, although it contains very little genetic information. Chromosomes 2 and 3 are large and metacentric; chromosome 4 is a very small acrocentric chromosome. In the salivary glands, the chromosomes are very large (see p. 323 in Chapter 11), making *Drosophila* an excellent subject for chromosome studies. In 2000, the complete genome of *D. melanogaster* was sequenced, followed by the sequencing of the genome of *D. pseudoobscura* in 2005 and the genomes of 10 additional *Drosophila* genomes in 2007. *Drosophila* continues today to be one of the most versatile and powerful of all genetic model organisms. ■

The Bacterium *Escherichia coli*

The most widely studied prokaryotic organism and one of the best genetically characterized of all the species is the bacterium *Escherichia coli*. Although some strains of *E. coli* are toxic and cause disease, most are benign and reside naturally in the intestinal tracts of humans and other warm-blooded animals. *E. coli* was first described by Theodore Escherich in 1885, but for many years, the assumption was that all bacteria reproduced only asexually and that genetic crosses were impossible. In 1946, Joshua Lederberg and Edward Tatum demonstrated that *E. coli* undergoes a type of sexual reproduction; their finding initiated the use of *E. coli* as a model genetic organism. A year later, Lederberg published the first genetic map of *E. coli* based on recombination frequencies, and in 1952, William Hays showed that mating between bacteria is asymmetrical, with one bacterium serving as genetic donor and the other as genetic recipient.

ADVANTAGES OF *E. COLI* AS A MODEL GENETIC ORGANISM

Escherichia coli is one of the true workhorses of genetics; its twofold advantage is rapid reproduction and small size. Under optimal conditions, this organism can reproduce every 20 minutes, and in a mere 7 hours, a single bacterial cell can give rise to more than 2

million descendants. One of the values of rapid reproduction is that enormous numbers of cells can be grown quickly, so even very rare mutations will appear in a short period. Consequently, numerous mutations in *E. coli*, affecting everything from colony appearance to drug resistance, have been isolated and characterized.

Escherichia coli is easy to culture in the laboratory in a liquid medium (see Figure 9.1a) or on a solid medium within petri plates (see Figure 9.1b). In liquid culture, *E. coli* cells will grow to a concentration of a billion cells per milliliter, and trillions of bacterial cells can be easily grown in a single test tube. When *E. coli* cells are diluted and spread onto the solid medium of a petri dish, individual bacteria reproduce asexually, giving rise to a concentrated clump of 10 million to 100 million genetically identical cells, called a colony. This colony formation makes it easy to isolate genetically pure strains of the bacteria.

THE *E. COLI* GENOME

The *E. coli* genome is on a single chromosome and—compared with those of humans, mice, plants, and other multicellular organisms—is relatively small, consisting of 4,638,858 base pairs. If stretched out straight, the DNA molecule in the single *E. coli* chromosome would be 1.6 mm long, almost a thousand times as long as the *E. coli* cell within which it resides (see Figure 11.1). To accommodate this huge amount of DNA within the confines of a single cell, the *E. coli*

chromosome is highly coiled and condensed. The information within the *E. coli* chromosome is also compact, having little noncoding DNA between and within the genes and having few sequences for which there is more than one copy. The *E. coli* genome contains an estimated 4300 genes, many of which have no known function. These "orphan genes" may play important roles in adapting to unusual environments, coordinating metabolic pathways, organizing the chromosome, or communicating with other bacterial cells. The haploid genome of *E. coli* makes it easy to isolate mutations because there are no dominant genes at the same loci to suppress and mask recessive mutations.

LIFE CYCLE OF *E. COLI*

Wild-type *E. coli* is prototrophic and can grow on a minimal medium that contains only glucose and some inorganic salts. Under most conditions, *E. coli* divides about once an hour, although in a richer medium containing sugars and amino acids, it will divide every 20 minutes. It normally reproduces through simple binary fission, in which the single chromosome of a bacterium replicates and migrates to opposite sides of the cell, followed by cell division, giving rise to two identical daughter cells (see Figure 2.5). Mating between bacteria, called conjugation, is controlled by fertility genes normally located on the F plasmid (see p. 263). In conjugation, one bacterium donates genetic material to another bacterium, followed by genetic recombination that integrates new alleles into the

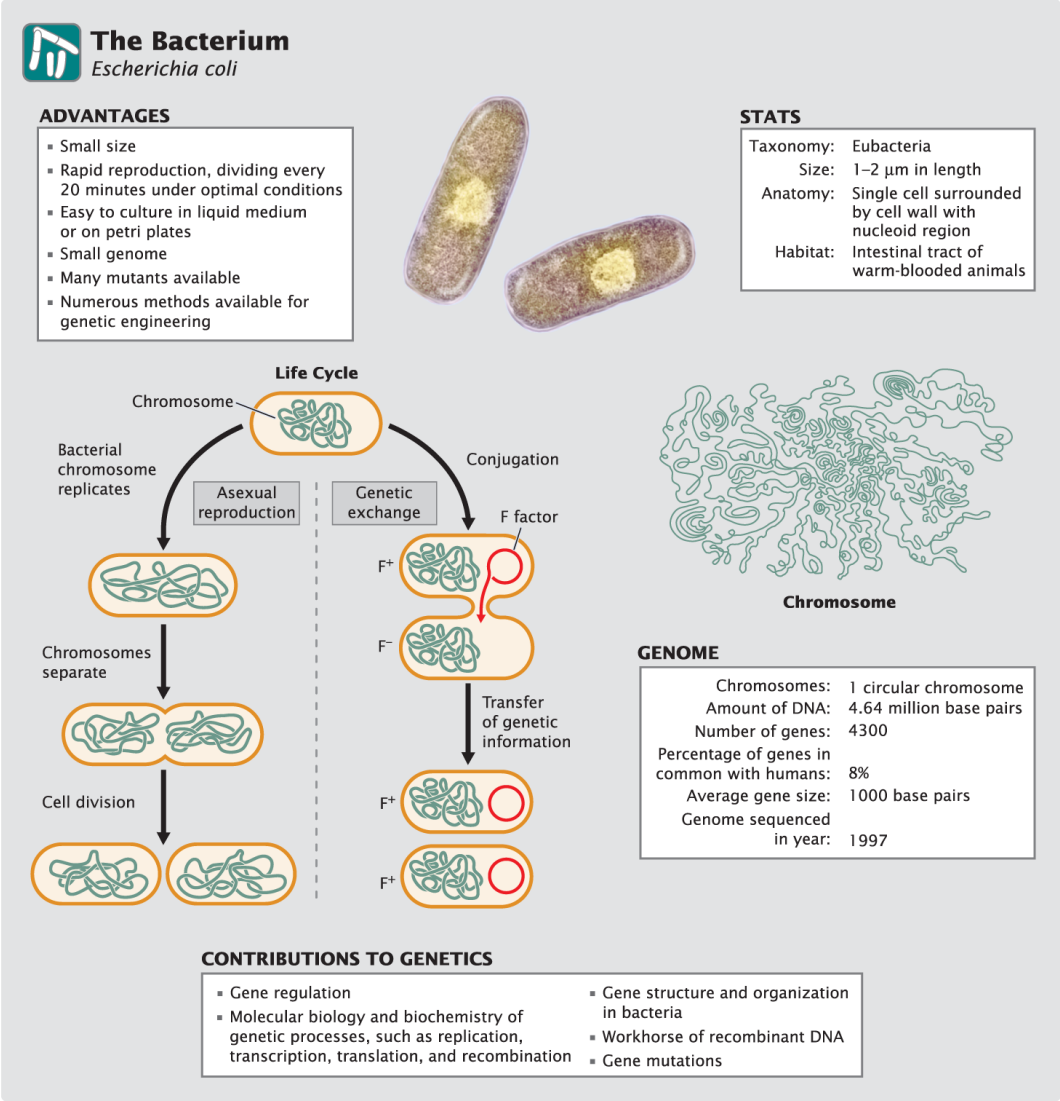
bacterial chromosome. Genetic material can also be exchanged between strains of *E. coli* through transformation and transduction (see Figure 9.7).

GENETIC TECHNIQUES WITH *E. COLI*

Escherichia coli is used in a number of experimental systems in which fundamental genetic processes are studied in detail. For example, in vitro translation systems contain within a test tube all the components necessary to translate the genetic information of a messenger RNA molecule into a polypeptide chain. Similarly, in vitro systems containing components from *E. coli* cells allow transcription, replication, gene expression, and many other important genetic functions to be studied and analyzed under controlled laboratory conditions.

Escherichia coli is also widely used in genetic engineering (recombinant DNA, see Chapter 19). Plasmids have been isolated from *E. coli* and genetically modified to create effective vectors for transferring genes into bacteria and eukaryotic cells. Often, new genetic constructs (DNA sequences created in the laboratory) are assembled and cloned in *E. coli* before transfer to other organisms. Methods have been developed to introduce specific mutations within *E. coli* genes, so genetic analysis no longer depends on the isolation of randomly occurring mutations. New DNA sequences produced by recombinant DNA can be introduced by transformation

into special strains of *E. coli* that are particularly efficient (competent) at taking up DNA.



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Description

The illustration shows two E. coli cells. The advantages are as follows: Small size; rapid reproduction, dividing every 20 minutes under optimal conditions; easy to culture in liquid medium or on petri plates; many mutants available; small genome; and numerous methods available for genetic engineering. Stats are as follows: Taxonomy: eubacteria; size: 1 to 2 micrometers in length; anatomy: single cell surrounded by cell wall with nucleoid region; habitat: Intestinal tract of warm-blooded animals. Chromosome shows a

scattered thread-like structure forming many spirals. Genome shows the following information: Chromosomes: 1 circular chromosome; amount of D N A: 4 point 64 million base pairs; number of genes: 4300; percentage of genes in common with humans: 8 percent; average gene size: 1000 base pairs; and genome sequenced in year: 19 97. Contributions to genetics are as follows: Gene regulation; molecular biology and biochemistry of genetic processes, such as replication, transcription, translation, and recombination; gene structure and organization in bacteria; workhorse of recombinant D N A; gene mutations. Life cycle is as follows: The life cycle starts with a bacterial cell with chromosome. It branches into two parts. The first part represents asexual reproduction and the second part represents genetic exchange. Bacterial chromosome replicates and the diagram shows a longer thread of chromosome than earlier. The chromosomes then separate into two parts and a constriction appears. Then, cell division takes place and two daughter cells are obtained. In the second part, conjugation occurs and an F-positive cell transmits F-factor to the F-negative cell. Transfer of genetic information takes place and two F-positive daughter cells are obtained.

Because of its powerful advantages as a model genetic organism, *E. coli* has played a leading role in many fundamental discoveries in genetics, including elucidating the genetic code, probing the nature of replication, and working out the basic mechanisms of gene regulation. ■

The Nematode Worm *Caenorhabditis elegans*

You may be asking: What is a nematode, and why is it a model genetic organism? Although rarely seen, nematodes are among the most abundant organisms on Earth, inhabiting soils throughout the world. Most are free living and cause no harm, but a few are important parasites of plants and animals, including humans. Although *Caenorhabditis elegans* has no economic or medical importance, it has become widely used in genetic studies because of its simple body plan, ease of culture, and high reproductive capacity. First introduced to the study of genetics by Sydney Brenner, who formulated plans in 1962 to use *C. elegans* for the genetic dissection of behavior, this species has made important contributions to the study of development, cell death, aging, and behavior.

ADVANTAGES OF *C. ELEGANS* AS A MODEL GENETIC ORGANISM

An ideal genetic organism, *C. elegans* is small, easy to culture, and produces large numbers of offspring. The adult *C. elegans* is about 1 mm in length. Most investigators grow *C. elegans* on agar-filled petri plates that are covered with a lawn of bacteria, which the nematodes devour. Thousands of worms can be easily cultured in a single laboratory. Compared with most multicellular animals, they have a

very short generation time, about 3 days at room temperature. They are also prolific reproducers, with a single female producing about 300 fertilized eggs in 3 to 4 days.

Another advantage of *C. elegans*, particularly for developmental studies, is that the worm is transparent, allowing easy observation of internal development at all stages. It has a simple body structure, with a small, invariant number of somatic cells: 959 cells in a mature hermaphroditic female and 1031 cells in a mature male.

LIFE CYCLE OF *C. ELEGANS*

Most mature adults are hermaphrodites, with the ability to produce both eggs and sperm and undergo self-fertilization. A few are male, which produce only sperm and mate with hermaphrodites. The hermaphrodites have two sex chromosomes (XX); the males possess a single sex chromosome (XO). Thus, hermaphrodites that self-fertilize produce only hermaphrodites (with the exception of a few males that result from nondisjunction of the X chromosomes). When hermaphrodites mate with males, half of the progeny are XX hermaphrodites and half are XO males.

Eggs are fertilized internally, either from sperm produced by the hermaphrodite or from sperm contributed by a male. The eggs are then laid, and development is completed externally. Approximately 14 hours after fertilization, a larva hatches from the egg and goes

through four larval stages—termed L1, L2, L3, and L4—that are separated by molts. The L4 larva undergoes a final molt to produce the adult worm. Under normal laboratory conditions, worms will live for 2 to 3 weeks.

THE *C. ELEGANS* GENOME

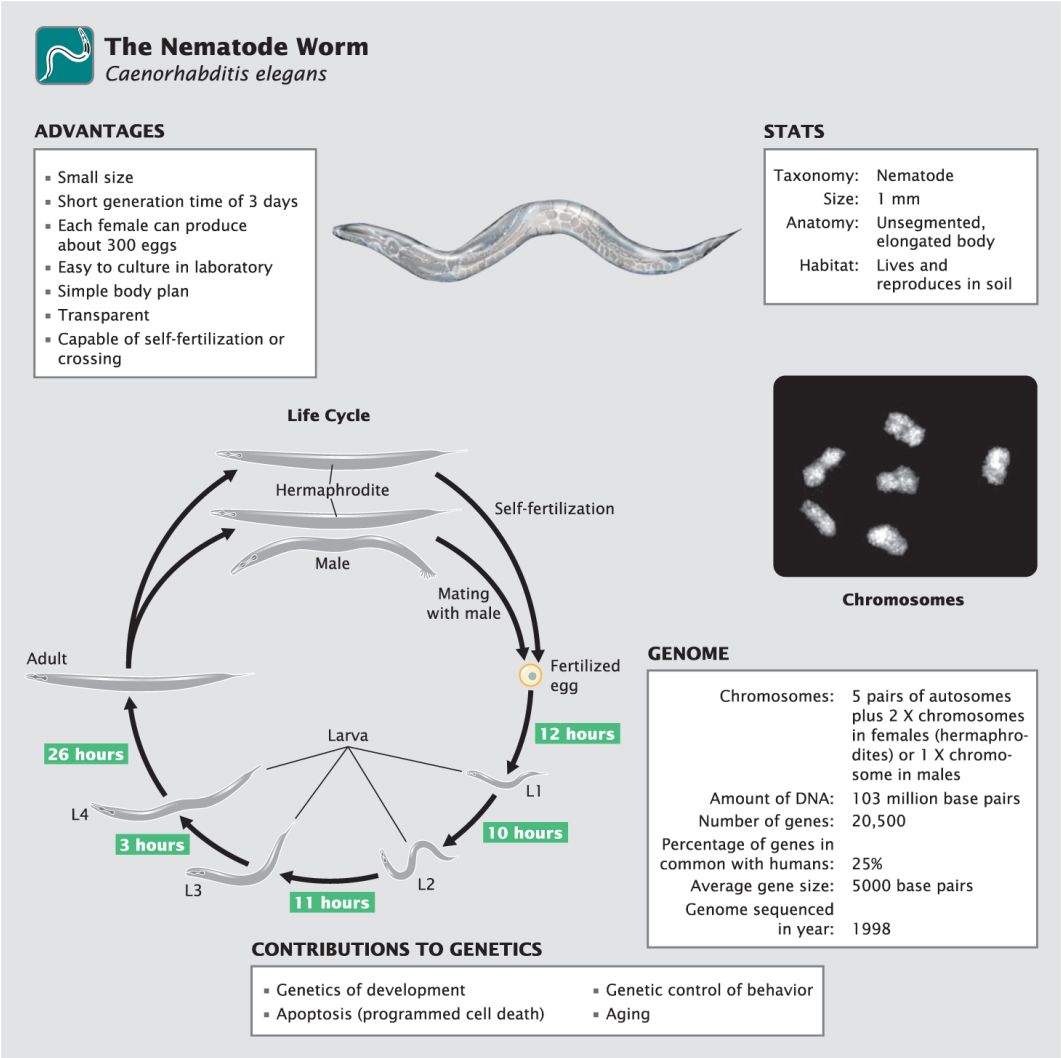
Geneticists began developing plans in 1989 to sequence the genome of *C. elegans*, and the complete genome sequence was obtained in 1998. Compared with the genomes of most multicellular animals, that of *C. elegans*, at 103 million base pairs of DNA, is small, which facilitates genomic analysis. The availability of the complete genome sequence provides a great deal of information about gene structure, function, and organization in this species. For example, the process of programmed cell death (apoptosis, see Chapter 22) plays an important role in development and in the suppression of cancer. Apoptosis in *C. elegans* is remarkably similar to that in humans. Having the complete genome sequence of *C. elegans*, and given its ease of genetic manipulation, geneticists have identified genes that participate in apoptosis, which has increased our understanding of apoptosis in humans and its role in cancer.

GENETIC TECHNIQUES WITH *C. ELEGANS*

Chemical mutagens are routinely used to generate mutations in *C. elegans—mutations* that are easy to identify and isolate. The ability of

hermaphrodites to self-fertilize means that progeny homozygous for recessive mutations can be obtained in a single generation; the existence of males means that genetic crosses can be carried out.

Developmental studies are facilitated by the transparent body of the worms. As stated earlier, *C. elegans* has a small and exact number of somatic cells. Researchers studying the development of *C. elegans* have meticulously mapped the entire cell lineage of the species, so the developmental fate of every cell in the adult body can be traced to the original single-celled fertilized egg. Developmental biologists often use lasers to destroy (ablate) specific cells in a developing worm and then study the effects on physiology, development, and behavior.



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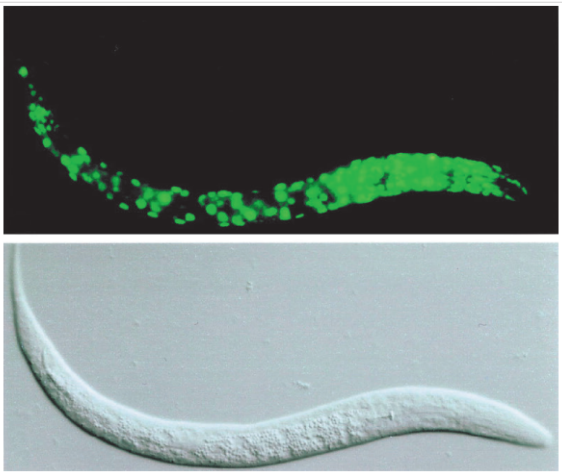
Photograph courtesy of William Goodyer and Monique Zetka.

Description

The illustration shows a C. elegans. The advantages are as follows: Small size, short generation time of 3 days, each female can produce about 300 eggs, simple body plan, easy to culture in laboratory, transparent, capable of self-fertilization or crossing. Stats are as follows: Taxonomy: Nematode; size: 1 m m; anatomy: Unsegmented, elongated body; habitat: Lives and reproduces in soil. A photo of chromosomes shows six short oval shaped bodies. Genome shows the following information: Chromosomes: 5 pairs of autosomes plus 2 X-chromosomes in females (hermaphrodites) or 1 X-chromosome in males; amount of D N A: 103 million base pairs; number of genes: 20,500; percentage of genes in common with humans: 25 percent; average gene size: 5000 base pairs; and genome sequenced in year: 19 98. Contributions to genetics are as follows: Genetics of

development, genetic control of behavior, apoptosis (programmed cell death), and aging. Life cycle is as follows: The life cycle starts with an adult worm either hermaphrodite or male. It undergoes either self-fertilization or mating with male respectively. A fertilized egg is formed. After 12 hours, larva L 1 forms. After 10 hours, larva L 2 forms. After 11 hours, larva L 3 forms and after 3 hours, larva L 4 forms. After 26 hours, this larva grows into an adult.

RNA interference has proved to be an effective tool for turning off genes in *C. elegans.* Geneticists inject double-stranded copies of RNA that is complementary to specific genes; the double-stranded RNA then silences the expression of these genes through the RNAi process. The worms can even be fed bacteria that have been genetically engineered to express the double-stranded RNA, thus avoiding the difficulties of microinjection.



Huaqi Jiang, Rong Guo, and Jo Anne Powell-Coffman. *The Caenorhabditis elegans* hif-1 gene encodes a bHLH- PAS protein that is required for adaptation to hypoxia. *PNAS* 98:7916–7921, 2001. ©2001 National Academy of Sciences, U.S.A.

Figure 1 A sequence for the green fluorescent protein (GFP) has been used to visually determine the expression of genes inserted into *C. elegans* (lower photograph). The gene for GFP is injected into the ovary of a worm and becomes

incorporated into the worm genome. The expression of this transgene produces GFP, which fluoresces green (upper photograph).

Description
In the first photograph, C. elegans is fluorescent stained and shows dots of green color. The second photo shows a white colored C. elegans.

Transgenic worms can be produced by injecting DNA into the ovary, where the DNA becomes incorporated into the oocytes. Geneticists have created a special reporter gene that produces the jellyfish green fluorescent protein (GFP). When this reporter gene is injected into the ovary and becomes inserted into the worm genome, its expression produces GFP, which fluoresces green, allowing the expression of the gene to be easily observed (Figure 1). ■

The Plant *Arabidopsis thaliana*

Much of the early work in genetics was carried out on plants, including Mendel's seminal discoveries in pea plants as well as important aspects of heredity, gene mapping, chromosome genetics, and quantitative inheritance in corn, wheat, beans, and other plants. However, by the mid-twentieth century, many geneticists had turned to bacteria, viruses, yeast, *Drosophila*, and mouse genetic models. Because a good genetic plant model did not exist, plants were relatively neglected, particularly for the study of molecular genetic processes.

This changed in the last part of the twentieth century with the widespread introduction of a new genetic model organism, the plant *Arabidopsis thaliana*. *A. thaliana* was identified in the sixteenth century, and the first mutant was reported in 1873, but this species was not commonly studied until the first detailed genetic maps appeared in the early 1980s. Today, *Arabidopsis* figures prominently in the study of genome structure, gene regulation, development, and evolution in plants, and it provides important basic information about plant genetics that is applied to economically important plant species.

ADVANTAGES OF *ARABIDOPSIS* AS A MODEL GENETIC ORGANISM

The thale-cress *Arabidopsis thaliana* is a member of the Brassicaceae family and grows as a weed in many parts of the world. Except in its role as a model genetic organism, *Arabidopsis* has no economic importance, but it has a number of characteristics that make it well-suited to the study of genetics. As an angiosperm, it has features in common with other flowering plants, some of which play critical roles in the ecosystem or are important sources of food, fiber, building materials, and pharmaceutical agents. *Arabidopsis*'s chief advantages are its small size (maximum height of 10–20 cm), prolific reproduction, and small genome.

Arabidopsis thaliana completes development—from seed germination to seed production—in about 6 weeks. Its small size and ability to grow under low illumination make it ideal for laboratory culture. Each plant is capable of producing from 10,000 to 40,000 seeds, and the seeds typically have a high rate of germination; thus, large numbers of progeny can be obtained from single genetic crosses.

THE *ARABIDOPSIS* GENOME

A key advantage for molecular studies is *Arabidopsis*'s small genome, which consists of only 125 million base pairs of DNA on five pairs of chromosomes, compared with 2.5 billion base pairs of DNA in the maize genome and 16 billion base pairs in the wheat genome. The genome of *A. thaliana* was completely sequenced in 2000, providing detailed information about gene structure and organization in this

species. A number of variants of *A. thaliana*—called ecotypes—that vary in shape, size, physiological characteristics, and DNA sequence are available for study.

LIFE CYCLE OF *ARABIDOPSIS*

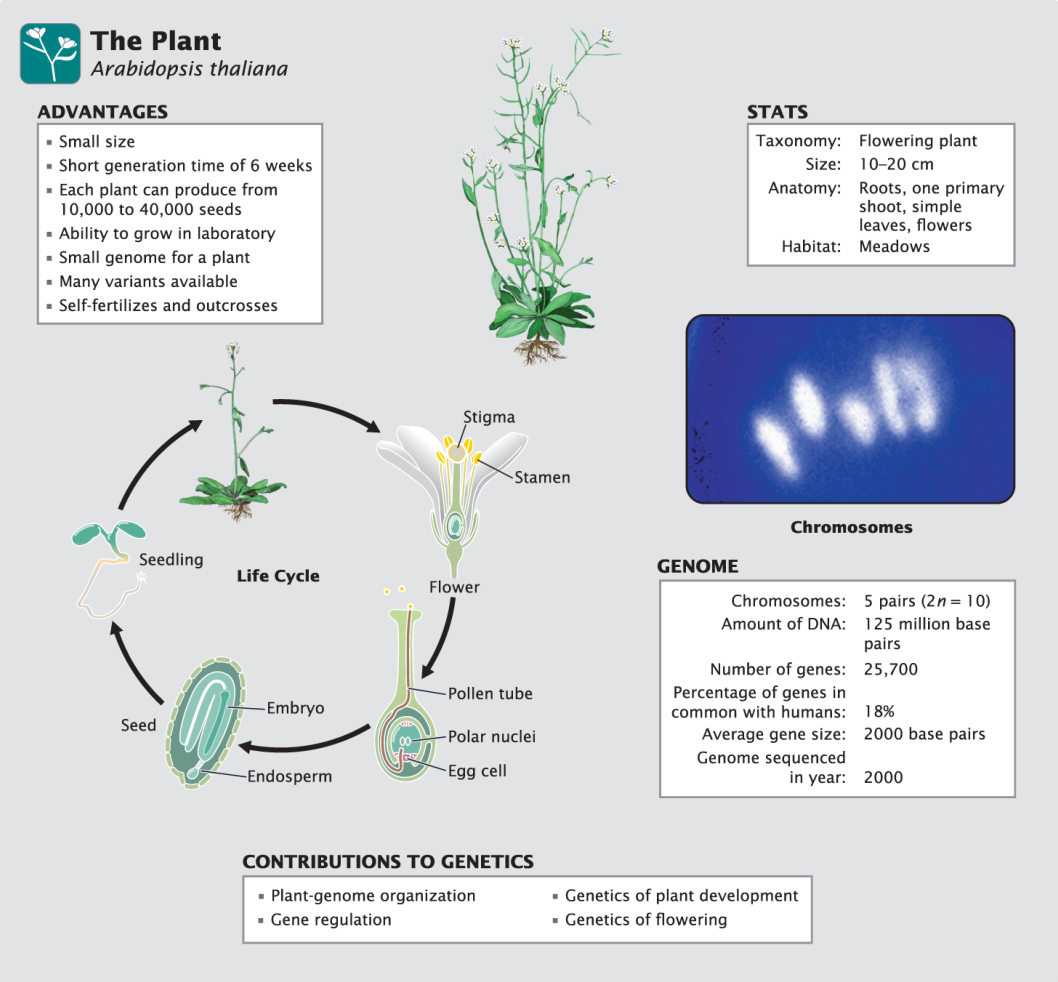
The *Arabidopsis* life cycle is fairly typical of most flowering plants (see Figure 2.22). The main vegetative part of the plant is diploid; haploid gametes are produced in the pollen and ovaries. When a pollen grain lands on the stigma of a flower, a pollen tube grows into the pistil and ovary. Two haploid sperm nuclei contained in each pollen grain travel down the pollen tube and enter the embryo sac. There, one of the haploid sperm cells fertilizes the haploid egg cell to produce a diploid zygote. The other haploid sperm cell fuses with two haploid nuclei to form the 3*n* endosperm, which provides tissue that will nourish the growing embryonic plant. The zygotes develop within the seeds, which are produced in a long pod.

Under appropriate conditions, the embryo germinates and begins to grow into a plant. The shoot grows upward and the roots downward, a compact rosette of leaves is produced, and, under the right conditions, the shoot enlarges and differentiates into flower structures. At maturity, *A. thaliana* is a low-growing plant with roots, a main shoot with branches that bear mature leaves, and small white flowers at the tips of the branches.

GENETIC TECHNIQUES WITH *ARABIDOPSIS*

A number of traditional and modern molecular techniques are commonly used with *Arabidopsis* and provide it with special advantages for genetic studies. *Arabidopsis* can self-fertilize, which means that any recessive mutation appearing in the germ line can be recovered in the immediate progeny. Cross-fertilization also is possible by removing the anther from one plant and dusting pollen on the stigma of another plant—essentially the same technique used by Gregor Mendel with pea plants (see Figure 3.5).

As already mentioned, many naturally occurring variants of *Arabidopsis* are available for study, and new mutations can be produced by exposing its seeds to chemical mutagens, radiation, or transposable elements that randomly insert into genes. The large number of offspring produced by *Arabidopsis* facilitates screening for rare mutations.



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company
Photograph courtesy of Anand P Tyagi and Luca Comai.

Description

The illustration shows an Arabidopsis thallana. The advantages are as follows: Small size, short generation time of 6 weeks, each plant can produce from 10,000 to 40,000 seeds, small genome for a plant, ability to grow in laboratory, many variants available, self-fertilizes and outcrosses. Stats are as follows: Taxonomy: Flowering plant; size: 10 to 20 c m; anatomy: Roots, one primary shoot, simple leaves, ¬flowers; habitat: meadows. Chromosomes show a picture of bright short rod shaped bodies. Genome shows the following information: Chromosomes: 5 pairs (2 n equals 10); amount of D N A: 125 million base pairs; number of genes: 25,700; percentage of genes in common with humans: 18 percent; average gene size: 2000 base pairs; and genome sequenced in year: 2000. Contributions to genetics are as follows: Plant-genome organization, genetics of plant development, gene regulation, and genetics of ¬flowering. The life cycle is as follows: The life cycle starts with an adult plant, which produces a flower with stigma and stamen. It

leads to an oval structure with a tube-like protuberance on top showing pollen above. The structure has a pollen tube inside the tube-like structure and polar nuclei and egg cell inside the oval-shaped structure. This leads to a seed with embryo and endosperm. Seedling forms after that, which leads to an adult plant.

Genes from other organisms can be transferred to *Arabidopsis* by the Ti plasmid from the bacterium *Agrobacterium tumefaciens*, which naturally infects plants and transfers the Ti plasmid to plant cells (see Chapter 19). After transfer to a plant cell, the Ti plasmid randomly inserts into the DNA of the plant that it infects, thereby generating mutations in the plant DNA in a process called *insertional mutagenesis.* Geneticists have modified the Ti plasmid to carry a *GUS* gene, which has no promoter of its own. The *GUS* gene encodes an enzyme that converts a colorless compound (X-Glu) into a blue dye. Because the *GUS* gene has no promoter, it is expressed only when inserted into the coding sequence of a plant gene. When that happens, the enzyme encoded by *GUS* is synthesized and converts X-Glu into a blue dye that stains the cell. This dye provides a means to visually determine the expression pattern of a gene that has been interrupted by Ti DNA, producing information about the expression of genes that are mutated by insertional mutagenesis. ■

The Mouse *Mus musculus*

The common house mouse, *Mus musculus*, is among the oldest and most valuable subjects for genetic study. It's an excellent genetic organism—small, prolific, and easy to keep, with a short generation time.

ADVANTAGES OF THE MOUSE AS A MODEL GENETIC ORGANISM

Foremost among many advantages that *Mus musculus* has as a model genetic organism is its close evolutionary relationship to humans. Being a mammal, the mouse is genetically, behaviorally, and physiologically more similar to humans than are other organisms used in genetic studies, making the mouse the model of choice for many studies of human and medical genetics. Other advantages include a short generation time compared with that of most other mammals. *Mus musculus* is well-adapted to life in the laboratory and can be easily raised and bred in cages that require little space; thus, several thousand mice can be raised within the confines of a small laboratory room. Mice have large litters (8–10 pups) and are docile and easy to handle. Finally, a large number of mutations have been isolated and studied in captive-bred mice, providing an important source of variation for genetic analysis.

LIFE CYCLE OF THE MOUSE

The production of gametes and reproduction in the mouse are very similar to those in humans. Diploid germ cells in the gonads undergo meiosis to produce sperm and oocytes, as outlined in Chapter 2. Male mice begin producing sperm at puberty and continue sperm production throughout the remainder of their lives. Starting at puberty, female mice go through an estrus cycle about every 4 days. If mating takes place during estrus, sperm are deposited into the vagina and swim into the oviduct, where one sperm penetrates the outer layer of the ovum, and the nuclei of sperm and ovum fuse. After fertilization, the diploid embryo implants into the uterus. Gestation typically takes about 21 days. Mice reach puberty in about 5 to 6 weeks and will live for about 2 years. A complete generation can be completed in about 8 weeks.

THE MOUSE GENOME

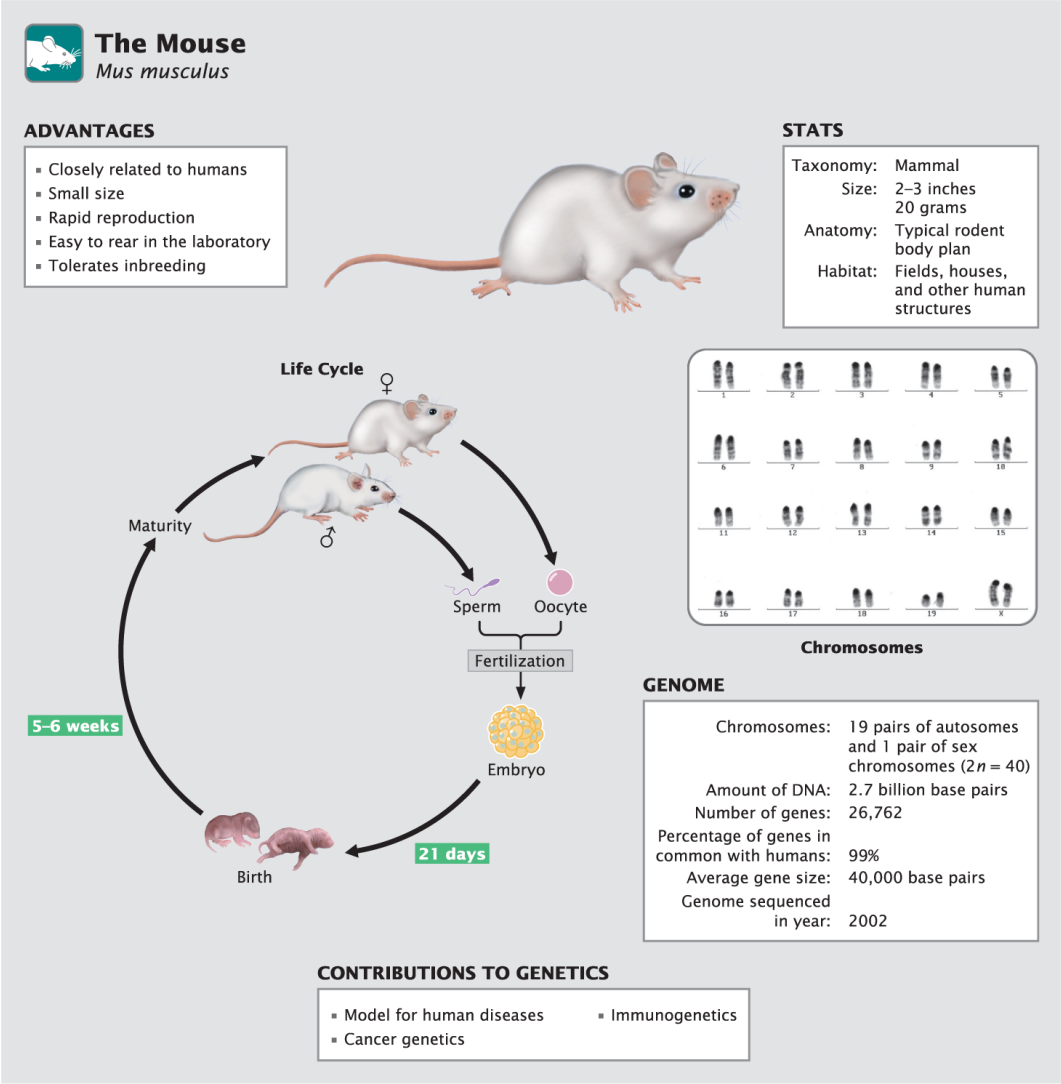
The mouse genome contains about 2.7 billion base pairs of DNA, which is similar in size to the human genome. Mice and humans also have similar numbers of genes. For most human genes, there are homologous genes in the mouse. An important tool for determining the function of an unknown gene in humans is to search for a homologous gene whose function has already been determined in the mouse. Furthermore, the linkage relations of many mouse genes are similar to those in humans, and the linkage relations of genes in mice often provide important clues to linkage relations among genes

in humans. The mouse genome is distributed across 19 pairs of autosomes and 1 pair of sex chromosomes.

GENETIC TECHNIQUES WITH THE MOUSE

A number of powerful techniques have been developed for use in the mouse. They include the creation of transgenic mice by the injection of DNA into a mouse embryo, the ability to disrupt specific genes by the creation of knockout mice, and the ability to insert specific sequences into specific loci (see Chapter 19). These techniques are made possible by the ability to manipulate the mouse reproductive cycle, including the ability to hormonally induce ovulation, isolate unfertilized oocytes from the ovary, and implant fertilized embryos back into the uterus of a surrogate mother. The ability to create transgenic, knockout, and knock-in mice has greatly facilitated the study of human genetics, and these techniques illustrate the power of the mouse as a model genetic organism.

Mouse and human cells can be fused, allowing somatic-cell hybridization techniques (see Chapter 7) that have been widely used to assign human genes to specific chromosomes. Mice also tolerate inbreeding well, and inbred strains of mice are easily created by brother–sister mating. Members of an inbred strain are genetically very similar or identical, allowing researchers to examine the effects of environmental factors on a trait.



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company
Photograph courtesy of Ellen C. Akeson and Muriel T. Davisson, The Jackson Laboratory, Bar Harbor, Maine.

Description

The illustration shows a mouse. The advantages are as follows: Closely related to humans, small size, rapid reproduction, easy to rear in the laboratory, and tolerates inbreeding. Stats are as follows: Taxonomy: Mammal; size: 2 to 3 inches, 20 grams; anatomy: Typical rodent body plan; habitat: Fields, houses, and other human structures. Chromosomes show a picture of 19 pairs of autosomes and a pair of X-chromosomes. Genome shows the following information: Chromosomes: 19 pairs of autosomes and 1 pair of sex chromosomes (2 n equals 40); amount of D N A: 2 point 7 million base pairs; number of genes: 26,762; percentage of genes in common with humans: 99 percent; average gene size: 40,000 base pairs; and genome sequenced in year: 2002. Contributions to genetics are as follows: Model for human diseases, immunogenetics, and

cancer genetics. The life cycle is as follows: The life cycle starts with a male and a female mouse. The male produces sperm and the female produces oocyte and both of these fertilize and produce an embryo. After 21 days, birth takes place and maturity leads to an adult male or female after 5 to 6 weeks.

The use of mice as a model genetic organism has led to many important genetic discoveries. In the early twentieth century, mice were used to study the genetic basis of coat-color variation in mammals. More recently, they have figured prominently in research on the genetic basis of cancer, and potential carcinogens are often tested in mice. Mice have been used to study genes that influence mammalian development, including mutations that produce birth defects in humans. A large number of mouse models of specific human diseases have been created—in some cases, by isolating and inbreeding mice with naturally occurring mutations, and in other cases, by using knockout and knock-in techniques to disable and modify specific genes. ■

The Yeast *Saccharomyces cerevisiae*

Common baker's yeast *(Saccharomyces cerevisiae)* has been widely adopted as a simple model system for the study of eukaryotic genetics. Long used for baking bread and making beer, yeast has more recently been used for the production of biofuels. Louis Pasteur identified *S. cerevisiae* as the microorganism responsible for fermentation in 1857, and its use in genetic analysis began about 1935. Having been the subject of extensive studies in classical genetics for many years, yeast genes are well-known and characterized. At the same time, yeast's unicellular nature makes it amenable to molecular techniques developed for bacteria. Thus, yeast combines both classical genetics and molecular biology to provide a powerful model for the study of eukaryotic genetic systems.

ADVANTAGES OF YEAST AS A MODEL GENETIC ORGANISM

The great advantage of yeast is that it not only is a eukaryotic organism, with genetic and cellular systems similar to those of other, more complex eukaryotes, such as humans, but also is unicellular, with many of the advantages of manipulation found with bacterial systems. Like bacteria, yeast cells require little space, and large numbers of cells can be grown easily and inexpensively in the laboratory.

Yeast exists in both diploid and haploid forms. When haploid, the cells possess only a single allele at each locus, which means that the allele will be expressed in the phenotype; unlike the situation in diploids, there is no dominance through which some alleles mask the expression of others. Therefore, recessive alleles can be easily identified in haploid cells, and then the interactions between alleles can be examined in the diploid cells.

Another feature that makes yeast a powerful genetic model system is that subsequent to meiosis, all the products of a meiotic division are present in a single structure called an ascus (see the next subsection) and remain separate from the products of other meiotic divisions. The four cells produced by a single meiotic division are termed a tetrad. In most organisms, the products of different meiotic divisions mix, so identification of the results of a single meiotic division is impossible. For example, if we were to isolate four sperm cells from the testes of a mouse, it is extremely unlikely that all four would have been produced by the same meiotic division. Having tetrads separate in yeast allows us to directly observe the effects of individual meiotic divisions on the types of gametes produced and to more easily identify crossover events. The genetic analysis of a tetrad is termed tetrad analysis.

Yeast has been subjected to extensive genetic analysis, and thousands of mutants have been identified. In addition, many powerful molecular techniques developed for manipulating genetic sequences in bacteria have been adapted for use in yeast. Yet in spite

of a unicellular structure and ease of manipulation, yeast cells possess many of the same genes found in humans and other complex multicellular eukaryotes, and many of these genes have identical or similar functions in these eukaryotes. Thus, the genetic study of yeast cells often contributes to our understanding of other, more complex eukaryotic organisms, including humans.

LIFE CYCLE OF YEAST

As stated earlier, *Saccharomyces cerevisiae* can exist as either haploid or diploid cells. Haploid cells usually exist when yeast is starved for nutrients and reproduce mitotically, producing identical haploid daughter cells through budding. Yeast cells can also undergo sexual reproduction. There are two mating types, a and α; haploid cells of different mating types fuse and then undergo nuclear fusion to create a diploid cell. The diploid cell is capable of budding mitotically to produce genetically identical diploid cells. Starvation induces the diploid cells to undergo meiosis, resulting in four haploid nuclei, which become separated into different cells, producing haploid spores. Because the four products of meiosis (a tetrad) are enclosed in a common structure—the ascus—all the products of a single meiosis can be isolated (tetrad analysis).

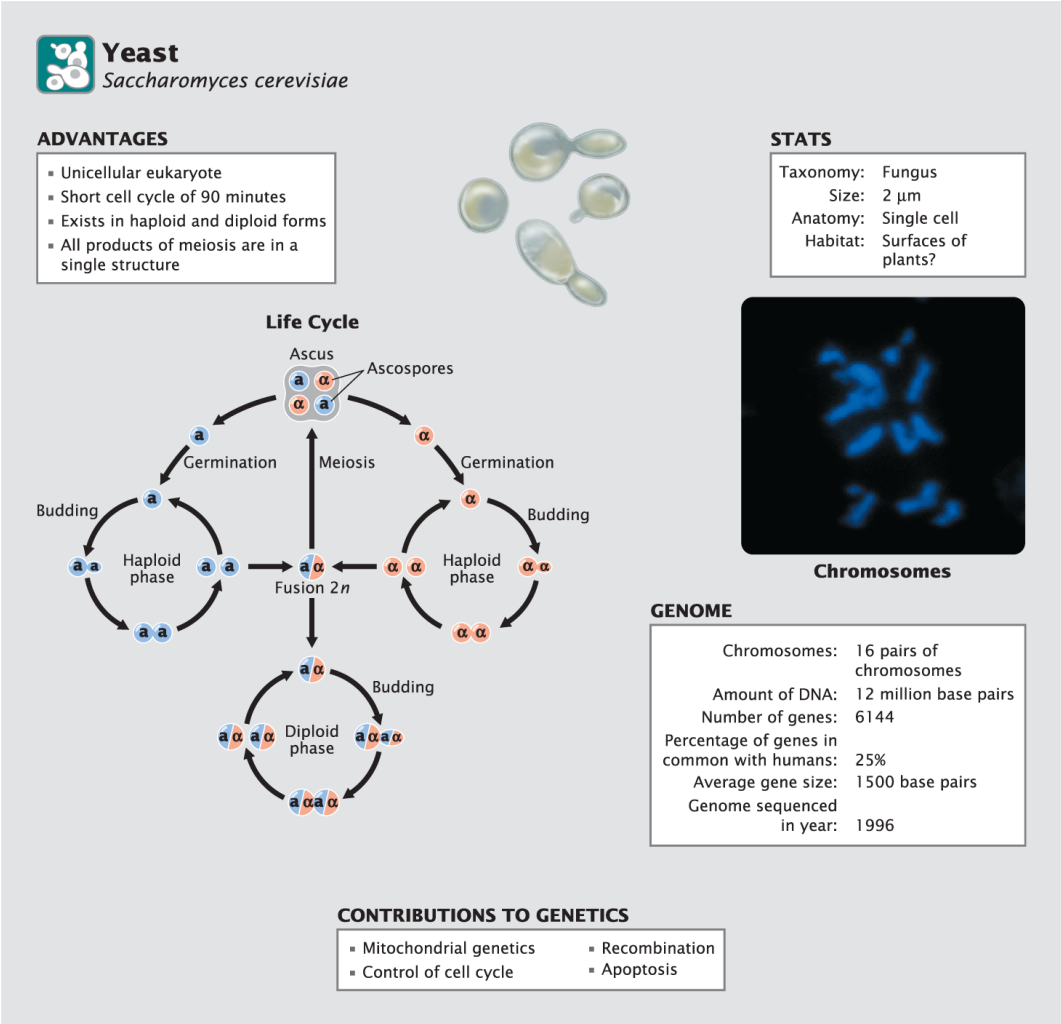
THE YEAST GENOME

Saccharomyces cerevisiae has 16 pairs of typical eukaryotic chromosomes. The rate of recombination is high, giving yeast a relatively long genetic map compared with those of other organisms. The genome of *S. cerevisiae* contains 12 million base pairs, plus the 2 million to 3 million base pairs of rRNA genes. In 1996, *S. cerevisiae* was the first eukaryotic organism whose genome was completely sequenced.

GENETIC TECHNIQUES WITH YEAST

One advantage of yeast to researchers is the use of plasmids to transfer genes or DNA sequences of interest into cells. Yeast cells naturally possesses a circular plasmid, named 2μ, that is 6300 bp long and is transmitted to daughter cells in mitosis and meiosis. This plasmid has an origin of replication recognized by the yeast replication system, so it replicates autonomously in the cell. The 2μ plasmid has been engineered to provide an efficient vector for transferring genes into yeast. In other cases, bacterial plasmids have been adapted for use in yeast. Some of them undergo homologous recombination with the yeast chromosome, transferring their sequences to the yeast chromosome. Shuttle vectors, which can be propagated in both bacteria and yeast, are particularly effective. Such vectors make it possible to construct and manipulate gene sequences in bacteria, where more powerful techniques are often available for genetic manipulation and selection, and then transfer

the gene sequences into yeast cells, where their function can be tested.



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company
Courtesy of Mara Stewart and Dean Dawson, Dawson Lab, Oklahoma Medical Research Foundation.

Description

The illustration shows four yeast cells, three of which are budding. The advantages are as follows: Unicellular eukaryote, short cell cycle of 90 minutes, exists in haploid and diploid forms, all products of meiosis are in a single structure. Stats are as follows: Taxonomy: Fungus; size: 2 micrometers; anatomy: single cell; habitat: surfaces of plants. Chromosomes show a picture of some fluorescent stained short chromosomes. Genome shows the following information: Chromosomes: 16 pairs of chromosomes; amount of D N A: 12 million base pairs; number of genes: 6144; percentage of genes in common with

humans: 25 percent; average gene size: 1500 base pairs; and genome sequenced in year: 19 96. Contributions to genetics are as follows: Mitochondrial genetics, control of cell cycle, recombination, and apoptosis. The life cycle is as follows: The life cycle starts with an ascus with four ascospores: two a and two alpha. An alpha ascospore and an ascospore germinate and go into haploid phase separately producing two alpha-alpha and two a-a, respectively through budding. Both of which can fuse and lead to a-alpha fusion 2 n. This leads to a diploid phase and forms two a-alpha cells through budding. This further produces ascospores.

Plasmids are limited in the size of DNA fragments that they can carry (see pp. 582–584 in Chapter 19). Yeast artificial chromosomes (YACs)—engineered DNA fragments that contain centromeric and telomeric sequences and segregate like chromosomes in meiosis and mitosis—overcome this limitation; YACs can hold DNA fragments as large as several hundred thousand base pairs. ■

Working with Fractions: A Review

Fractions are commonly used in working genetics problems because we are often concerned with probabilities and with the proportions of progeny resulting from genetic crosses. At some point in the past, you undoubtedly learned how to manipulate fractions, but perhaps that was a long time ago. The following brief review is designed to help you recall some basic operations with fractions.

Characteristics of Fractions

What is a fraction? You probably remember that a fraction is used to represent a proportion, or part of a whole. It is a number presented in the form $\frac{a}{b}$, sometimes written as $^{a}/_{b}$. The top part of the fraction (the part above the line, a in this case) is called the **numerator**. The bottom part of the fraction (the part below the line, b in this case) is called the **denominator**.

We can think of the fraction $\frac{a}{b}$ as a out of b, as in 3 out of 4($\frac{3}{4}$) of the progeny of a cross have yellow seeds. Another way to think of the fraction $\frac{a}{b}$ is that it means a divided by b. For example, $\frac{3}{4} = 3 \div 4 = 0.75$. We can always convert a fraction into a decimal number by simply dividing the numerator by the denominator.

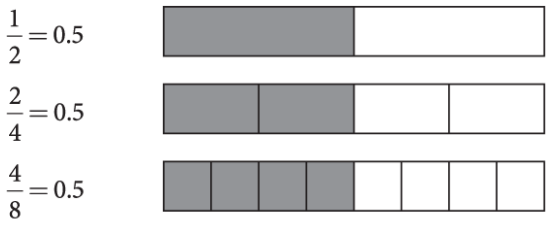
In simple fractions, which are the kind most often used in genetics problems, *a* and *b* are integers—positive or negative whole numbers. A simple fraction might be $\frac{3}{4}$ or $\frac{7}{125}$, but not $\frac{0.7}{1.5}$. Also, remember that the denominator of a fraction cannot be zero because the fraction $\frac{a}{b}$ means that *a* is divided by *b*, and a number cannot be divided by zero. You will probably also remember that any number divided by itself is 1, so any fraction with the same number as the numerator and the denominator is equal to 1:

$$\frac{2}{2} = 1.0$$
$$\frac{7}{7} = 1.0$$
$$\frac{16}{16} = 1.0$$

The size of a fraction depends on both the numerator and the denominator. If the numerators of two fractions are the same but the denominators differ, the fraction with the larger denominator is smaller. Thus, $\frac{3}{8}$ $(= 0.375)$ is smaller than $\frac{3}{4}$ $(= 0.750)$. The opposite is true of the numerator. If the denominators of two fractions are the same but the numerators differ, the fraction with the larger numerator is larger. For example, $\frac{14}{16}$ $(= 0.875)$ is larger than $\frac{3}{16}$ $(= 0.188)$.

Equivalent Fractions

Now that we've discussed some of the features of fractions, let's review equivalent fractions. The same number can be represented by many different fractions. For example, the fractions $\frac{1}{2}$, $\frac{2}{4}$, and $\frac{4}{8}$ all equal 0.5. These fractions, which have different numbers in the numerator and the denominator but are numerically equivalent, are called equivalent fractions. The proportions represented by equivalent fractions are the same:



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Description
The first fraction is 1 over 2 equals 0 point 5. Beside this, a rectangular block is shown divided into two equal parts; one part is shaded. The second fraction is 2 over 4 equals 0 point 5. Beside this, a rectangular block is shown divided into four equal parts; two parts are shaded. The third fraction is 4 over 8 equals 0 point 5. Beside this, a rectangular block is shown divided into eight equal parts; four parts are shaded. All the rectangular blocks are of same size.

We can produce equivalent fractions by multiplying both the numerator and the denominator by the same number, which is the same as multiplying by one:

$$\frac{1}{2} = \frac{1 \times 2}{2 \times 2} = \frac{2}{4} = 0.5$$

$$\frac{2}{4} = \frac{2 \times 2}{4 \times 2} = \frac{4}{8} = 0.5$$

In this way, we can create fractions that have the same denominator. This ability will be important when we need to add and subtract fractions (see Adding and Subtracting Fractions below). For example, suppose we want to convert $\frac{1}{3}$ and $\frac{1}{4}$ into equivalent fractions, both with the same denominator. We could multiply $\frac{1}{3}$ by $\frac{4}{4}$ and $\frac{1}{4}$ by $\frac{3}{3}$, so that both proportions now have 12 in the denominator:

$$\frac{1}{3} = \frac{1 \times 4}{3 \times 4} = \frac{4}{12}$$

$$\frac{1}{4} = \frac{1 \times 3}{4 \times 3} = \frac{3}{12}$$

The equivalent fraction $\frac{4}{12}$ is equal in value to the original fraction $(\frac{1}{3})$ but has 12 in the denominator. Likewise, $\frac{3}{12}$ is equal in value to the original fraction $(\frac{1}{4})$ but also has 12 in the denominator.

Simplifying Fractions

We often want to put fractions into their simplest equivalent forms. Simplifying fractions, for example, may help us to see that two fractions are equivalent.

To simplify a fraction, the numerator and denominator must first be factored. A factor is a number that the original number can be

divided by evenly with no remainder. For example, 4 is a factor of 12 because 4 will divide into 12 exactly 3 times ($\frac{12}{4} = 3$). However, 5 is not a factor of 12 because 5 cannot divide into 12 evenly ($\frac{12}{5} = 2.4$). Thus, 4 can be factored as 4 = 2 × 2. Similarly, 8 can be factored as 8 = 2 × 2 × 2, and 15 can be factored as 15 = 3 × 5.

Fractions can be simplified when the numerator and denominator have a common factor:

$$\frac{4}{16} = \frac{4 \times 1}{4 \times 4}$$

Because $\frac{4}{4} = 1$, the 4s in both the numerator and denominator cancel out:

$$\frac{4}{16} = \frac{\cancel{4} \times 1}{\cancel{4} \times 4} = \frac{1}{4}$$

Thus, $\frac{4}{16}$ can be simplified to $\frac{1}{4}$.

Now that we've discussed some features of fractions, let's review how we carry out simple mathematical operations with fractions, such as addition, subtraction, multiplication, and division. These are the primary operations you will need to use as you work genetics problems.

Adding and Subtracting Fractions

Adding and subtracting fractions is easy, but first they must have the same denominator. To add two or more fractions with the same denominator, add the numerators and place the sum over the common denominator:

$$\frac{1}{4} + \frac{1}{4} = \frac{2}{4}$$

$$\frac{3}{8} + \frac{2}{8} + \frac{1}{8} = \frac{6}{8}$$

Similarly, to subtract two or more fractions with the same denominator, subtract the numerators and place the result over the common denominator:

$$\frac{6}{8} - \frac{1}{8} = \frac{5}{8}$$
$$\frac{13}{16} - \frac{3}{16} - \frac{1}{16} = \frac{9}{16}$$

When the fractions you want to add or subtract don't have the same denominator, then you must convert them into equivalent fractions that have the same denominator (see Equivalent Fractions above). For example, to add $\frac{1}{2}$ and $\frac{1}{3}$, convert both fractions into equivalent fractions with the common denominator 6:

$$\frac{1}{2} = \frac{1 \times 3}{2 \times 3} = \frac{3}{6}$$

$$\frac{1}{3} = \frac{1 \times 2}{3 \times 2} = \frac{2}{6}$$

Once the fractions have the same denominator, they can be added by summing the numerators and putting the result over the common denominator:

$$\frac{3}{6} + \frac{2}{6} = \frac{5}{6}$$

The same is true when subtracting fractions—they must have the same denominator. For example, to subtract $\frac{1}{3}$ from $\frac{7}{8}$, first convert the two fractions into equivalent fractions that have a common denominator, such as 24:

$$\frac{7}{8} = \frac{7 \times 3}{8 \times 3} = \frac{21}{24}$$

$$\frac{1}{3} = \frac{1 \times 8}{3 \times 8} = \frac{8}{24}$$

Then subtract the numerators and put the result over the common denominator:

$$\frac{21}{24} - \frac{8}{24} = \frac{13}{24}$$

Multiplying and Dividing Fractions

Multiplying two or more fractions is simple. You multiply the numerators of the fractions and then multiply the denominators of the fractions:

$$\frac{1}{2} \times \frac{3}{4} = \frac{1 \times 3}{2 \times 4} = \frac{3}{8}$$

$$\frac{3}{4} \times \frac{3}{4} = \frac{3 \times 3}{4 \times 4} = \frac{9}{16}$$

$$\frac{1}{2} \times \frac{3}{4} \times \frac{3}{4} = \frac{1 \times 3 \times 3}{2 \times 4 \times 4} = \frac{9}{32}$$

Dividing fractions is a bit more complicated. Suppose we want to divide one fraction by another fraction, such as $\frac{1}{4}$ divided by $\frac{3}{4}$. First, take the reciprocal of the divisor (the second fraction, or $\frac{3}{4}$). Taking the reciprocal means that you reverse the numbers in the numerator and the denominator. For example, the reciprocal of $\frac{3}{4}$ is $\frac{4}{3}$. Once you have the reciprocal, multiply the dividend (the first fraction, or $\frac{1}{4}$) by the reciprocal of the divisor:

$$\frac{1}{4} \times \frac{4}{3} = \frac{4}{12}$$

Another way to think about dividing fractions is to represent the original division ($\frac{1}{4}$ divided by $\frac{3}{4}$) as a fraction itself, with $\frac{1}{4}$ as the numerator and $\frac{3}{4}$ as the denominator:

$$\frac{\frac{1}{4}}{\frac{3}{4}}$$

To carry out the division, you multiply both the numerator and the denominator by the reciprocal of the denominator ($\frac{4}{3}$), which is the equivalent of multiplying by 1:

$$\frac{\frac{1}{4} \times \frac{4}{3}}{\frac{3}{4} \times \frac{4}{3}} = \frac{\frac{4}{12}}{\frac{12}{12}}$$

The denominator now equals $\frac{12}{12}$, which is 1, so the equation simplifies to

$$\frac{\frac{4}{12}}{\frac{12}{12}} = \frac{\frac{4}{12}}{1} = \frac{4}{12}$$

This is the same result we obtained when we multiplied the dividend by the reciprocal of the divisor.

Practice Problems

1. What is the denominator in the following fraction: $\frac{6}{16}$? What is the numerator?
2. What is the decimal value of the following fraction: $\frac{8}{32}$?

3. Which of the following fractions represents a larger number, $\frac{7}{16}$ or $\frac{9}{16}$?
4. Which of the following fractions represents a larger number, $\frac{10}{32}$ or $\frac{10}{64}$?
5. Convert the following fractions into equivalent fractions that have the same denominator:
   a. $\frac{1}{2}$ and $\frac{2}{3}$
   b. $\frac{4}{7}$ and $\frac{5}{9}$
   c. $\frac{3}{4}$ and $\frac{7}{12}$
6. Simplify the following fractions:
   a. $\frac{6}{9}$
   b. $\frac{10}{25}$
   c. $\frac{4}{64}$
   d. $\frac{12}{32}$
7. Add or subtract the following fractions:
   a. $\frac{5}{8} + \frac{2}{8}$
   b. $\frac{15}{16} - \frac{9}{16}$
   c. $\frac{2}{3} + \frac{1}{16}$
   d. $\frac{53}{64} - \frac{5}{16}$
8. Multiply the following fractions:
   a. $\frac{3}{4} \times \frac{1}{8}$
   b. $\frac{3}{16} \times \frac{2}{3}$

c. $\frac{3}{8} \times \frac{13}{32}$

9. Divide the following fractions:
   a. $\frac{1}{4}$ by $\frac{1}{2}$
   b. $\frac{3}{4}$ by $\frac{3}{8}$
   c. $\frac{1}{16}$ by $\frac{7}{8}$

Answers to Practice Problems

1. Denominator = 16; numerator = 6
2. 0.25
3. $\frac{9}{16}$
4. $\frac{10}{32}$
5. a. $\frac{1}{2} = \frac{1 \times 3}{2 \times 3} = \frac{3}{6}; \frac{2}{3} = \frac{2 \times 2}{3 \times 2} = \frac{4}{6}$
   b. $\frac{4}{7} = \frac{4 \times 9}{7 \times 9} = \frac{36}{63}; \frac{5}{9} = \frac{5 \times 7}{9 \times 7} = \frac{35}{63}$
   c. $\frac{3}{4} = \frac{3 \times 3}{4 \times 3} = \frac{9}{12}; \frac{7}{12}$
6. a. $\frac{6}{9} = \frac{3 \times 2}{3 \times 3} = \frac{2}{3}$
   b. $\frac{10}{25} = \frac{5 \times 2}{5 \times 5} = \frac{2}{5}$
   c. $\frac{4}{64} = \frac{4 \times 1}{4 \times 16} = \frac{1}{16}$
   d. $\frac{12}{32} = \frac{4 \times 3}{4 \times 8} = \frac{3}{8}$
7. a. $\frac{7}{8}$
   b. $\frac{6}{16}$, which simplifies to $\frac{3}{8}$

c. $\frac{2}{3} = \frac{2 \times 16}{3 \times 16} = \frac{32}{48}; \frac{1}{16} = \frac{1 \times 3}{16 \times 3} = \frac{3}{48}; \frac{32}{48} + \frac{3}{48} = \frac{35}{48}$

d. $\frac{5}{16} = \frac{5 \times 4}{16 \times 4} = \frac{20}{64}; \frac{53}{64} - \frac{20}{64} = \frac{33}{64}$

8. a. $\frac{3}{4} \times \frac{1}{8} = \frac{3}{32}$

b. $\frac{3}{16} \times \frac{2}{3} = \frac{6}{48}$, which simplifies to $\frac{1}{8}$

c. $\frac{3}{8} \times \frac{13}{32} = \frac{39}{256}$

9. a. $\frac{1}{4} \times \frac{2}{1} = \frac{2}{4}$, which simplifies to $\frac{1}{2}$

b. $\frac{3}{4} \times \frac{8}{3} = \frac{24}{12}$, which simplifies to 2

c. $\frac{1}{16} \times \frac{8}{7} = \frac{8}{112}$, which simplifies to $\frac{1}{14}$

Glossary

abortive initiation
Process during initiation of transcription in which RNA polymerase repeatedly generates and releases short transcripts, from 2 to 6 nucleotides in length, while still bound to the promoter. Occurs in both prokaryotes and eukaryotes.

acentric chromatid
Chromatid that lacks a centromere; produced when crossing over takes place within a paracentric inversion. The acentric chromatid does not attach to a spindle microtubule and does not segregate in meiosis or mitosis, so it is usually lost after one or more rounds of cell division.

acrocentric chromosome
Chromosome in which the centromere is near one end, producing a long arm at one end and a knob, or satellite, at the other end.

adaptive mutation
Process by which a specific environment induces mutations that enable organisms to adapt to that environment.

addition rule
Rule stating that the probability of any of two or more mutually exclusive events is calculated by adding the probabilities of the events.

additive genetic variance
Component of genetic variance that comprises the additive effects of genes on the phenotype.

adenine (A)
Purine base in DNA and RNA.

adjacent-1 segregation
Type of segregation that takes place in a heterozygote for a translocation. If the original, nontranslocated chromosomes are N_1 and N_2 and the chromosomes containing the translocated segments are T_1 and T_2, then adjacent-1 segregation takes place when N_1 and T_2 move toward one pole and T_1 and N_2 move toward the opposite pole.

adjacent-2 segregation
Type of segregation that takes place in a heterozygote for a translocation. If the original, nontranslocated chromosomes are N_1 and N_2 and the chromosomes containing the translocated segments are T_1 and T_2, then adjacent-2 segregation takes place when N_1 and T_1 move toward one pole and T_2 and N_2 move toward the opposite pole.

A-DNA
Right-handed helical structure of DNA that exists when little water is present.

affinity capture
Use of an antibody to capture one protein from a complex mixture of proteins. The captured protein will "pull down" with it any proteins with which it physically interacts, which can then be analyzed by mass spectrometry to identify the proteins.
allele
One of two or more alternative forms of a gene.
allelic frequency
Proportion of a particular allele within a population.
allopatric speciation
Speciation that occurs when a geographic barrier splits a population into two groups and blocks the exchange of genes between them.
allopolyploidy
Condition in which all chromosomes of a polyploid individual are from two or more species.
allosteric protein
Protein that changes its conformation upon binding to another molecule.
alternate segregation
Type of segregation that takes place in a heterozygote for a translocation. If the original, nontranslocated chromosomes are N_1 and N_2 and the chromosomes containing the translocated segments are T_1 and T_2, then alternate segregation takes place when N_1 and N_2 move toward one pole and T_1 and T_2 move toward the opposite pole.
alternation of generations
Complex life cycle in plants that alternates between the diploid sporophyte stage and the haploid gametophyte stage.
alternative processing pathway
One of several pathways by which a single pre-mRNA can be processed in different ways to produce alternative types of mRNA.
alternative splicing
Process in which a single pre-mRNA can be spliced in more than one way to produce different types of mRNA.
Ames test
Test in which special strains of bacteria are used to evaluate the potential of chemicals to cause cancer.
amino acid
Repeating unit of proteins; each consists of a central carbon atom bonded to an amino group, a hydrogen atom, a carboxyl group, and an R group.
aminoacyl (A) site
One of the three sites in a ribosome occupied by a tRNA during translation. All charged tRNAs (with the exception of the initiator tRNA) first enter the A site.
aminoacyl-tRNA synthetase

Enzyme that attaches an amino acid to a tRNA. Each aminoacyl-tRNA synthetase recognizes a particular amino acid.

amniocentesis

Prenatal testing of fetal tissue that involves obtaining a sample of amniotic fluid from the uterus of a pregnant woman.

amphidiploidy

Type of allopolyploid in which two different diploid genomes are combined such that every chromosome has one and only one homologous partner and the genome is functionally diploid.

anagenesis

Evolution taking place within a single lineage.

anaphase

Stage of mitosis in which sister chromatids separate and move toward opposite spindle poles.

anaphase I

Stage of meiosis I in which homologous chromosomes separate and move toward the spindle poles.

anaphase II

Stage of meiosis II in which sister chromatids separate and the chromatids are pulled to opposite poles.

aneuploidy

Change in the number of individual chromosomes; most often an increase or a decrease of one or two chromosomes.

annotation

Linking of a gene's sequence information to other information about the gene's function and expression, about the protein it encodes, and about similar genes in other species.

Antennapedia complex

Cluster of five homeotic genes in fruit flies that affects the development of the adult fly's head and anterior thoracic segments.

antibody

Protein produced by a B cell that circulates in the blood and other body fluids. An antibody binds to a specific antigen and marks the antigen for destruction by phagocytic cells.

anticipation

Phenomenon in which a genetic trait becomes more strongly expressed, or is expressed at an earlier age, as it is passed from generation to generation.

anticodon

Sequence of three nucleotides in tRNA that pairs with the corresponding codon on mRNA to ensure that the amino acids link in the correct order during translation.

antigen

A molecule (usually a protein) that is recognized by the immune system and elicits an immune reaction.

antigenic drift
The appearance of new strains of a rapidly evolving virus because of mutations.
antigenic shift
Major change in a viral genome through the reassortment of genetic material from two different strains of the virus.
antiparallel
The orientation of the two polynucleotide strands of the DNA double helix in opposite directions.
antisense RNA
Small RNA molecule that base pairs with a complementary DNA or RNA sequence and affects its functioning.
antiterminator
Protein or DNA sequence that prevents the termination of transcription.
apoptosis
Programmed cell death, in which a cell's DNA is degraded, its nucleus and cytoplasm shrink, and the cell undergoes phagocytosis by other cells without leakage of its contents.
archaea
One of the three primary divisions of life, consisting of unicellular organisms with prokaryotic cell structure.
artificial selection
Selection practiced by humans.
attenuation
Type of gene regulation in some bacterial operons in which transcription begins at the transcription start site but terminates prematurely, before the RNA polymerase even reaches the structural genes.
attenuator
Secondary structure that forms in the $5'$ untranslated region of some operons and causes the premature termination of transcription.
autoimmune disease
Disease that results from the body producing an immune reaction to its own antigens.
autonomous element
Transposable element that is fully functional and able to transpose on its own.
autonomously replicating sequence (ARS)
DNA sequence that confers the ability to replicate; contains an origin of replication.
autopolyploidy
Condition in which all the sets of chromosomes of a polyploid individual are derived from a single species.
autosome
Chromosome that is the same in males and females; a nonsex chromosome.
auxotrophic

Possessing a nutritional mutation that disrupts the ability to synthesize an essential biological molecule; an auxotrophic bacterium cannot grow on a minimal medium but can grow on a minimal medium to which has been added the biological molecule that it cannot synthesize.

backcross

Cross between an F_1 individual and either of the parental genotypes.

bacteria

One of the three primary divisions of life, consisting of prokaryotic unicellular organisms; also called **eubacteria**.

bacterial artificial chromosome (BAC)

Cloning vector originally constructed from the F plasmid that can hold very large fragments of DNA (as long as 300,000 bp).

bacterial colony

See **colony**.

bacteriophage

Virus that infects bacterial cells.

balancing selection

Natural selection that maintains genetic variation.

Barr body

Inactivated X chromosome that appears as a condensed, darkly staining body in the nuclei of most cells of female placental mammals.

basal transcription apparatus

Complex of transcription factors, RNA polymerase, and other proteins that assemble on the promoter and are capable of initiating minimal levels of transcription.

base

See **nitrogenous base**.

base analog

Chemical substance that has a structure similar to that of one of the four standard nitrogenous bases of DNA and may be incorporated into newly synthesized DNA molecules in replication.

base-excision repair

DNA repair that first excises modified bases and then replaces the entire nucleotide.

base substitution

Mutation in which a single pair of bases in DNA is altered.

B cell

Type of lymphocyte that produces humoral immunity; matures in the bone marrow and produces antibodies.

B-DNA

Right-handed helical structure of DNA that exists when water is abundant; the secondary structure described by Watson and Crick and probably the most common DNA structure in cells.

behavioral isolation

Reproductive isolation due to differences in behavior that prevent interbreeding.

ß sliding clamp

A ring-shaped polypeptide component of DNA polymerase III that encircles DNA and allows the polymerase to slide easily along the DNA template strand during replication.

bidirectional replication

Replication at both ends of a replication bubble.

bioinformatics

Interdisciplinary field that combines molecular biology and computer science; develops databases, computer-search algorithms, gene-prediction software, and other analytical tools that are used to make sense of DNA-, RNA-, and protein-sequence data.

biological species concept

Definition of a species as a group of organisms whose members are capable of interbreeding with one another but are reproductively isolated from the members of other species. Because different species do not exchange genes, each species evolves independently. Not all biologists adhere to this concept.

biotechnology

Use of biological processes, particularly molecular genetics and recombinant DNA technology, to produce products of commercial value.

bithorax complex

Cluster of three homeotic genes in fruit flies that influences the adult fly's posterior thoracic and abdominal segments.

bivalent

A homologous pair of synapsed chromosomes consisting of four chromatids; also called a **tetrad**.

blending inheritance

Early concept of heredity proposing that the traits of offspring are a blend, or mixture, of parental traits.

branch

An evolutionary connection between nodes in a phylogenetic tree representing an evolutionary connection between organisms.

branch migration

Movement of a cross bridge along two DNA molecules.

branch point

Adenine nucleotide in nuclear pre-mRNA introns that lies 18 to 40 nucleotides upstream of the $3'$ splice site.

broad-sense heritability

Proportion of phenotypic variance that can be attributed to genetic variance.

cap-binding complex (CBC)

A group of proteins in eukaryotes that binds to the 5′ cap and initiates translation. Aids in exporting mRNA from the nucleus and then promotes the initial (pioneer) round of translation in the cytoplasm.

carcinogen

A substance capable of causing cancer.

caspase

Enzyme that cleaves other proteins and regulates apoptosis. Each caspase is synthesized as a large, inactive precursor (a procaspase) that is activated by cleavage, often by another caspase.

Cas proteins

Proteins that function in CRISPR-Cas systems. Combine with crRNA to form an effector complex that cleaves DNA at specific base sequences.

catabolite activator protein (CAP)

Protein that functions in catabolite repression. When bound with cAMP, CAP binds to the promoters of certain operons and stimulates transcription.

catabolite repression

System of gene control in some bacterial operons in which glucose is used preferentially and the metabolism of other sugars is repressed in the presence of glucose.

cDNA (complementary DNA) library

Collection of bacterial colonies or phages containing DNA fragments that have been produced by reverse transcription of cellular mRNA.

cell cycle

Stages through which a cell passes from one cell division to the next.

cell line

Genetically identical cells that divide indefinitely and can be cultured in the laboratory.

cell theory

Theory stating that all life is composed of cells, that cells arise only from preexisting cells, and that the cell is the fundamental unit of structure and function in living organisms.

cellular immunity

Type of immunity resulting from T cells, which recognize antigens found on the surfaces of the body's own cells.

centiMorgan (cM)

See **map unit**.

central dogma

Concept that genetic information passes from DNA to protein in a one-way information pathway.

centriole

Cytoplasmic organelle consisting of microtubules; present at each pole of the spindle apparatus in animal cells.

centromere

Constricted region on a chromosome that stains less strongly than the rest of the chromosome; serves as the attachment point for spindle microtubules.

centrosome

Structure from which the spindle apparatus develops; contains the centriole.

Chargaff's rules

Rules developed by Erwin Chargaff and his colleagues concerning the ratios of bases in DNA.

checkpoint

A key transition point at which progression to the next stage in the cell cycle is regulated.

chiasma (pl., chiasmata)

Point of attachment between homologous chromosomes at which crossing over takes place.

chi-square goodness-of-fit test

Statistical test used to evaluate how well a set of observed values fit the expected values. The probability associated with a calculated chi-square value is the probability that the difference between the observed and the expected values is due to chance.

chloroplast DNA (cpDNA)

DNA in chloroplasts; has many characteristics in common with eubacterial DNA and typically consists of a circular molecule that lacks histone proteins and encodes some of the rRNAs, tRNAs, and proteins found in chloroplasts.

chorionic villus sampling (CVS)

Procedure used for prenatal genetic testing in which a small piece of the chorion (the outer layer of the placenta) is removed from a pregnant woman. A catheter is inserted through the vagina and cervix into the uterus; suction is then applied to remove the sample.

chromatin

Material found in the eukaryotic nucleus; consists of DNA and histone proteins.

chromatin-remodeling complex

Complex of proteins that bind directly to particular sites on DNA and reposition the nucleosomes, allowing other transcription factors and RNA polymerase to bind to promotors and initiate transcription.

chromatin-remodeling protein

Protein that binds to a DNA sequence and disrupts chromatin structure, causing the DNA to become more accessible to RNA polymerase and other proteins.

chromosome

Structure consisting of DNA and associated proteins that carries and transmits genetic information.

chromosome deletion

Loss of a chromosome segment.

chromosome duplication

Mutation that doubles a segment of a chromosome.

chromosome inversion

Rearrangement in which a segment of a chromosome has been inverted 180 degrees.

chromosome jumping

Method of locating a gene by moving from a gene on a cloned fragment to sequences on distantly linked fragments.

chromosome mutation

Variations in the number and structure of chromosomes; often affects many genes and has large phenotypic effects.

chromosome puff

Localized swelling of a polytene chromosome; a region of chromatin in which DNA has unwound and is undergoing transcription.

chromosome rearrangement

Chromosome mutations that change the structures of individual chromosomes.

chromosome theory of heredity

Theory stating that genes are located on chromosomes.

chromosome walking

Method of locating a gene by using partly overlapping genomic clones to move in steps from a previously cloned, linked gene to the gene of interest.

circular RNA (circRNA)

Circular RNA molecules found in eukaryotic cells. Some contain binding sites for miRNAs.

cis configuration

See **coupling configuration**.

cladogenesis

Evolution in which one lineage is split into two.

clonal evolution

Process by which mutations that enhance the ability of cells to proliferate predominate in a clone of cells, allowing the clone to become increasingly rapid in growth and increasingly aggressive in proliferation properties.

cloning vector

Stable, replicating DNA molecule to which a foreign DNA fragment can be attached for introduction into a cell.

cloverleaf

Secondary structure with four major arms common to all tRNAs.

codominance

Type of allelic interaction in which the heterozygote simultaneously expresses the phenotypes of both homozygotes.

codon

Sequence of three nucleotides that encodes one amino acid in a protein.

coefficient of coincidence

Ratio of observed double crossovers to expected double crossovers.

cohesin
A protein that holds the two sister chromatids of a chromosome together. The breakdown of cohesin at the centromeres enables the chromatids to separate in anaphase of mitosis and anaphase II of meiosis.

cohesive end
Short, single-stranded overhanging end on a DNA molecule produced when the DNA is cut by certain restriction enzymes; also called a sticky end. Cohesive ends are complementary and can spontaneously pair to rejoin DNA fragments that have been cut with the same restriction enzyme.

colinearity
Concept of direct correspondence between the nucleotide sequence of a gene and the continuous sequence of amino acids in a protein.

colony
Clump of genetically identical bacteria derived from a single bacterial cell that undergoes repeated rounds of division.

comparative genomics
Field of genomics that studies similarities and differences among genomes of different organisms.

competence
The ability to take up DNA from the environment (to be transformed).

complementary DNA strands
Nucleotide strands of DNA in which each purine on one strand pairs with a specific pyrimidine on the opposite strand (A pairs with T, and G pairs with C).

complementation
Manifestation of two different mutations in the heterozygous condition as the wild-type phenotype; indicates that the mutations are at different loci.

complementation test
Test designed to determine whether two different mutations are at the same locus (are allelic) or at different loci (are nonallelic). Two individuals that are homozygous for two independently derived mutations are crossed, producing F_1 progeny that are heterozygous for the mutations. If the mutations are at the same locus, the F_1 will have a mutant phenotype. If the mutations are at different loci, the F_1 will have a wild-type phenotype.

complete dominance
Type of dominance in which the same phenotype is expressed in homozygotes (*AA*) and in heterozygotes (*Aa*); only the dominant allele is expressed in a heterozygote.

complete linkage
Linkage between genes that are located close together on the same chromosome with no crossing over between them.

complete medium
A medium that contains all the substances required by bacteria for growth and reproduction. Auxotrophic mutants can grow on a complete medium.

composite transposon
Type of transposable element in bacteria that consists of two insertion sequences flanking a segment of DNA.
compound heterozygote
An individual who carries two different alleles at a locus that result in a recessive phenotype.
concept of dominance
Principle of heredity discovered by Mendel stating that when two different alleles are present in a genotype, only one of them—the dominant allele—is observed in the phenotype.
concordance
Percentage of twin pairs that are concordant for a particular trait.
concordant
Refers to a pair of twins who both have a particular trait.
condensins
A group of proteins that bind to chromosomes as a cell enters prophase, causing the chromosomes to become more compact and visible under a light microscope.
conditional mutation
Mutation that is expressed only under certain conditions.
conditional probability
Probability that is modified by additional information.
conjugation
Mechanism by which genetic material can be transferred from one bacterium to another. In conjugation, two bacteria lie close together, and a connection forms between them. A plasmid, or sometimes a part of the bacterial chromosome, passes through this connection from one cell to the other.
consanguinity
Mating between closely related people.
consensus sequence
Sequence that comprises the most commonly encountered nucleotides found at a specific location in DNA or RNA.
−10 consensus sequence (Pribnow box)
Consensus sequence (TATAAT) found in most bacterial promoters approximately 10 bp upstream of the transcription start site.
−35 consensus sequence
Consensus sequence (TTGACA) found in many bacterial promoters approximately 35 bp upstream of the transcription start site.
constitutive
Describes a gene that is expressed continually without regulation.
constitutive mutation
A mutation that causes the continuous transcription of one or more structural genes.

contig
Set of two or more overlapping DNA fragments that have been assembled in the correct order to form a continuous stretch of DNA sequence.
continuous characteristic
Characteristic that displays a large number of possible phenotypes that are not easily distinguished, such as human height.
continuous replication
Replication of the leading strand of DNA in the same direction as that of unwinding, allowing new nucleotides to be added continuously to the $3'$ end of the new strand as the template is exposed.
coordinate induction
The simultaneous synthesis of several proteins stimulated by a specific molecule, the inducer.
copy-number variation (CNV)
Difference among individual organisms in the number of copies of any large DNA sequence (larger than 1000 bp).
core enzyme
Five subunits at the heart of most bacterial RNA polymerases that, during transcription, catalyze the elongation of the RNA molecule by the addition of RNA nucleotides; consists of two copies of a subunit called alpha (α) and single copies of subunits beta (β), beta prime (β′), and omega (ω).
corepressor
Substance that inhibits transcription in a repressible system of gene regulation; usually a small molecule that binds to a repressor protein and alters it so that the repressor is able to bind to DNA and inhibit transcription.
core promoter
The DNA sequence located immediately upstream of a eukaryotic gene, and the site to which the basal transcription apparatus binds.
correlation
Degree of association between two or more variables.
correlation coefficient
Statistic that measures the degree of association between two or more variables. A correlation coefficient can range from −1 to +1. A positive value indicates a direct relation between the variables; a negative correlation indicates an inverse relation between the variables. The absolute value of the correlation coefficient provides information about the strength of association between the variables.
cosmid
Cloning vector consisting of a plasmid packaged in a viral protein coat that can be transferred to bacteria by viral infection; can carry large pieces of DNA into bacteria.
cotransduction
Process in which two or more genes are transferred together from one bacterial cell to another by a virus. Only genes located close together on a bacterial chromosome will be cotransduced.

cotransformation
Process in which two or more genes are taken up together during cell transformation.
coupling (cis) configuration
Arrangement of linked genes in which wild-type alleles of two or more genes are found on one chromosome, and mutant alleles are on the homologous chromosome.
CpG island
DNA region that contains many copies of a cytosine base followed by a guanine base; often found near transcription start sites in eukaryotic DNA. The cytosine bases in CpG islands are commonly methylated when genes are inactive but are demethylated before the initiation of transcription.
CRISPR array
A series of repeated DNA sequences called clustered regularly interspaced short palindromic repeats located in the bacterial or archaeal chromosome. Fragments of foreign DNA are inserted into the array; these are later transcribed into CRISPR RNA.
CRISPR-Cas system
A system used by bacteria and archaea as a defense against foreign DNA. Has been adapted for use as a molecular tool for precisely cutting DNA, providing a powerful way of editing the genome.
CRISPR RNAs (crRNAs)
Small RNA molecules found in prokaryotes that assist in the destruction of foreign DNA molecules.
cross bridge
In a heteroduplex DNA molecule, the point at which each nucleotide strand passes from one DNA molecule to the other.
crossing over
Exchange of genetic material between homologous but nonsister chromatids.
C-value
Amount of DNA per cell in an organism.
C-value paradox
The absence of a relation between the C-values (genome sizes) of eukaryotes and organismal complexity.
cyclin
A key protein in the control of the cell cycle; combines with a cyclin-dependent kinase (CDK). The levels of cyclin rise and fall in the course of the cell cycle.
cyclin-dependent kinase (CDK)
A key protein in the control of the cell cycle; combines with cyclin.
cytokinesis
Process by which the cytoplasm of a cell divides.
cytoplasmic inheritance
Inheritance of characteristics encoded by genes located in the cytoplasm. Because the cytoplasm is usually contributed entirely by one parent, most cytoplasmically inherited characteristics are inherited from only one parent.

cytosine (C)
Pyrimidine base in DNA and RNA.
deamination
Loss of an amino group (NH_2) from a base.
degenerate genetic code
Refers to the fact that the genetic code contains more codons than are needed to specify all 20 common amino acids.
deletion
Mutation in which one or more nucleotides are deleted from a DNA sequence.
deletion mapping
Technique for determining the chromosomal location of a gene by studying the association of its phenotype or product with particular chromosome deletions.
denaturation
Process that separates the strands of double-stranded DNA when DNA is heated.
deoxyribonucleotide
Basic building block of DNA, consisting of deoxyribose, a phosphate group, and a nitrogenous base.
deoxyribose
Five-carbon sugar in DNA; lacks a hydroxyl group on the $2'$-carbon atom.
depurination
Break in the covalent bond connecting a purine base to the $1'$-carbon atom of deoxyribose sugar, resulting in the loss of the purine base.
determination
Process by which a cell becomes committed to developing into a particular cell type.
diakinesis
Fifth substage of prophase I in meiosis. In diakinesis, chromosomes contract, the nuclear membrane breaks down, and the spindle forms.
dicentric bridge
Structure produced when the two centromeres of a dicentric chromatid are pulled toward opposite poles, stretching the dicentric chromosome across the center of the nucleus. Eventually, the dicentric bridge breaks as the two centromeres are pulled farther apart.
dicentric chromatid
Chromatid that has two centromeres; produced when crossing over takes place within a paracentric inversion. The two centromeres of the dicentric chromatid are frequently pulled toward opposite poles in mitosis or meiosis, breaking the chromosome.
dideoxyribonucleoside triphosphate (ddNTP)
Special substrate for DNA synthesis used in the Sanger dideoxy sequencing method; identical with dNTP (the usual substrate for DNA synthesis) except that it lacks a $3'$-OH group. The incorporation

of a ddNTP into DNA terminates DNA synthesis.

dihybrid cross

Cross between two individuals that differ in two characteristics—more specifically, a cross between individuals that are homozygous for different alleles at two loci (*AA BB* × *aa bb*); also refers to a cross between two individuals that are both heterozygous at two loci (*Aa Bb* × *Aa Bb*).

dioecious

Belonging to a species in which an individual organism has either male or female reproductive structures.

diploid

Possessing two sets of chromosomes (two genomes).

diplotene

Fourth substage of prophase I in meiosis. In diplotene, centromeres of homologous chromosomes move apart, but the homologs remain attached at chiasmata.

directional selection

Selection in which one allele or trait is favored over another.

direct repair

DNA repair in which modified bases are changed back into their original structures.

direct-to-consumer genetic test

Test for a genetic condition that can be purchased directly by a consumer without the involvement of a physician or an other health-care provider.

discontinuous characteristic

Characteristic that exhibits only a few, easily distinguished phenotypes. An example is seed shape, in which seeds are either round or wrinkled.

discontinuous replication

Replication of the lagging strand of DNA in the direction opposite that of unwinding, which means that DNA must be synthesized in short stretches (Okazaki fragments).

discordant

Refers to one twin having a particular trait while the other twin does not.

displaced duplication

Chromosome rearrangement in which the duplicated segment is some distance from the original segment, either on the same chromosome or on a different one.

dizygotic twins

Nonidentical twins that arise when two separate eggs are fertilized by two different sperm.

D loop

Region of mitochondrial DNA that contains sites where replication and transcription of the mtDNA is initiated.

DNA fingerprinting

Technique used to identify individuals by examining their DNA sequences.

DNA gyrase

Topoisomerase enzyme in *E. coli* that relieves the torsional strain that builds up ahead of the replication fork.

DNA helicase

Enzyme that unwinds double-stranded DNA by breaking hydrogen bonds that exist between the bases of the two nucleotide strands of a DNA molecule.

DNA library

Collection of clones containing all the DNA fragments from one source.

DNA ligase

Enzyme that catalyzes the formation of a phosphodiester bond between adjacent 3′-OH and 5′-phosphate groups in a DNA molecule without adding another nucleotide to the strand.

DNA methylation

Modification of DNA by the addition of methyl groups by specific enzymes to certain positions on the bases.

DNA polymerase

Enzyme that synthesizes DNA.

DNA polymerase I

Bacterial DNA polymerase that removes RNA nucleotides of the primers and replaces them with DNA nucleotides.

DNA polymerase II

Bacterial DNA polymerase that takes part in DNA repair; restarts replication after synthesis has halted because of DNA damage.

DNA polymerase III

Bacterial DNA polymerase that synthesizes new nucleotide strands by adding new nucleotides to the 3′-OH group provided by the primer.

DNA polymerase IV

Bacterial DNA polymerase that probably takes part in DNA repair.

DNA polymerase V

Bacterial DNA polymerase that probably takes part in DNA repair.

DNA polymerase α

Eukaryotic DNA polymerase that initiates nuclear DNA synthesis.

DNA polymerase β

Eukaryotic DNA polymerase that participates in DNA repair.

DNA polymerase δ

Eukaryotic DNA polymerase that replicates the lagging strand during DNA synthesis; also carries out DNA repair and translesion DNA synthesis.

DNA polymerase ε

Eukaryotic DNA polymerase that replicates the leading strand during DNA synthesis.

DNA polymerase γ

Eukaryotic DNA polymerase that replicates mitochondrial DNA. A γ-like DNA polymerase replicates chloroplast DNA.

DNase I hypersensitive site

Chromatin region that becomes sensitive to digestion by the enzyme DNase I.

DNA sequencing

Process of determining the sequence of bases along a DNA molecule.

DNA transposon

Transposable element that transposes as DNA.

domain

Functional part of a protein.

dominance genetic variance

Component of genetic variance that can be attributed to dominance (interaction between genes at the same locus).

dominant

Refers to an allele or a phenotype that is expressed in homozygotes (*AA*) and in heterozygotes (*Aa*); only the dominant allele is expressed in a heterozygote phenotype.

dosage compensation

A mechanism to equalize the amount of protein produced by X-linked genes and autosomal genes. In placental mammals, dosage compensation is accomplished by the random inactivation of one X chromosome in the cells of females.

double fertilization

Fertilization in plants; includes the fusion of a sperm cell with an egg cell to form a zygote and the fusion of a second sperm cell with the polar nuclei to form an endosperm.

double-strand-break model

Model of homologous recombination in which a DNA molecule undergoes double-strand breaks.

Down syndrome (trisomy 21)

Human condition characterized by variable degrees of intellectual disability; characteristic facial features; slower growth and development; and an increased incidence of heart defects, leukemia, and other abnormalities. It is caused by the duplication of all or part of chromosome 21.

driver

Mutation that directly contributes to the development of cancer.

ecological isolation

Reproductive isolation in which different species live in different habitats and interact with the environment in different ways, so that their members do not encounter one another and do not reproduce with one another.

Edwards syndrome (trisomy 18)

Human condition characterized by severe intellectual disability, low-set ears, a short neck, deformed feet, clenched fingers, heart problems, and other disabilities; results from the presence of three copies of chromosome 18.

effective population size
Effective number of breeding adults in a population; influenced by the number of individuals contributing genes to the next generation, their sex ratio, variation between individuals in reproductive success, fluctuations in population size, age structure of the population, and whether mating is random.
egg
Female gamete.
egg-polarity genes
Set of genes that determine the major axes of development in an early fruit-fly embryo. One set of egg-polarity genes determines the anterior–posterior axis, and another determines the dorsal–ventral axis.
electrophoresis
See **gel electrophoresis**.
elongation factor G (EF-G)
Protein that combines with GTP and is required for movement of the ribosome along the mRNA during translation.
elongation factor Ts (EF-Ts)
Protein that regenerates elongation factor Tu in the elongation stage of translation.
elongation factor Tu (EF-Tu)
Protein taking part in the elongation stage of translation; joins with GTP and then with a charged tRNA to form a three-part complex and then delivers the charged tRNA to the ribosome.
endosymbiotic theory
Theory stating that some membrane-bounded organelles, such as mitochondria and chloroplasts, originated as free-living bacterial cells that entered into an endosymbiotic relation with a host cell and evolved into the present-day organelles; supported by a number of similarities in structure and sequence between organelle and bacterial DNA.
engineered nuclease
Protein consisting of the part of a restriction enzyme that cleaves DNA, combined with another protein that recognizes and binds to a specific DNA sequence; capable of making unique double-stranded cuts in DNA at predetermined sequences. Engineered nucleases can be custom designed to bind to and cut any particular DNA sequence.
enhancer
Sequence that stimulates maximal transcription of distant genes; affects only genes on the same DNA molecule (is cis acting), contains short consensus sequences, is not fixed in relation to the transcription start site, can stimulate promoters in its vicinity, and may be upstream or downstream of the gene. The function of an enhancer is independent of sequence orientation.
enhancer RNA (eRNA)
Type of noncoding RNA transcribed from enhancer sequences that may play a role in regulating the expression of protein-encoding genes.

environmental DNA (eDNA)
DNA detected in environmental samples; often used to determine the presence of rare and secretive organisms in the environment.
environmental variance
Component of phenotypic variance that is due to environmental differences among individual members of a population.
epialleles
Alleles that do not differ in their base sequence but have epigenetic differences that produce heritable variations in phenotypes.
epigenetic change
A stable alteration of chromatin structure that may be passed on to descendant cells or individuals. *See also* **epigenetics**.
epigenetic mark
Heritable change in DNA or chromatin structure that does not involve alteration of the base sequence and that brings about changes in gene expression.
epigenetics
Phenomena due to alterations in DNA that do not include changes in the base sequence; often affects the way in which DNA sequences are expressed. Such alterations are often stable and heritable in the sense that they are passed to descendant cells or individuals.
epigenome
All epigenetic modifications within the genome of an individual organism.
epigenome-wide association studies
Research that looks for associations between the presence of epigenetic changes at specific locations and the presence of individual phenotypes.
episome
Plasmid capable of replicating freely and able to integrate into a bacterial chromosome.
epistasis
Type of gene interaction in which a gene at one locus masks or suppresses the effects of a gene at a different locus.
epistatic gene
Gene that masks or suppresses the effect of a gene at a different locus.
equilibrium
Situation in which no further change takes place; in population genetics, refers to a population in which allelic frequencies do not change.
equilibrium density gradient centrifugation
Method used to separate molecules or organelles of different density by centrifugation.
eubacteria
See **bacteria**.
euchromatin

Chromatin that undergoes the normal process of condensation and decondensation in the course of the cell cycle.

eukaryote

One of the three primary divisions of life; an organism that has a compartmentalized cell structure, including a nuclear envelope and membrane-bounded organelles. Eukaryotes may be unicellular or multicellular.

evolution

Genetic change that takes place in a group of organisms.

exit (E) site

One of the three sites in a ribosome occupied by a tRNA during translation. In the elongation stage, the tRNA moves from the peptidyl P site to the E site, from which it then exits the ribosome.

exon

Coding region of a gene that is interrupted by introns; after transcription and posttranscriptional processing, the exons remain in mRNA.

exon shuffling

Process by which exons of different genes are exchanged, creating genes that are mosaics of other genes; has been important in the evolution of eukaryotic genes.

expanding nucleotide repeat

Type of mutation in which the number of copies of a set of nucleotides (most often three nucleotides) increases in succeeding generations.

expression vector

Cloning vector containing DNA sequences such as a promoter, a ribosome-binding site, and transcription initiation and termination sites that allow DNA fragments inserted into the vector to be transcribed and translated.

expressivity

Degree to which a trait is expressed.

familial Down syndrome

Human condition caused by a Robertsonian translocation in which the long arm of chromosome 21 is translocated to another chromosome; tends to run in families.

fertilization

Fusion of gametes (sex cells) to form a zygote.

F (fertility) factor

Episome of *E. coli* that controls conjugation and gene exchange between *E. coli* cells. The F factor contains an origin of replication and genes that enable the bacterium to undergo conjugation.

F_1 (first filial) generation

Offspring of the parents in the P generation in a genetic cross.

F_2 (second filial) generation

Offspring of the F_1 generation in a genetic cross; the third generation of a genetic cross.

first polar body

One of the products of meiosis I in oogenesis; the smaller cell, which contains half the chromosomes but only a small part of the cytoplasm.

fitness

Reproductive success of a genotype relative to that of other genotypes in a population.

5-methylcytosine

Modified form of the base cytosine, containing a methyl group (CH_3) on the $5'$ carbon.

5′ cap

Modified $5'$ end of eukaryotic mRNA, consisting of an extra modified nucleotide (7-methyl guanosine) and methyl groups at the $2'$ position of the ribose sugar in one or more subsequent nucleotides. Sometimes a methyl group is also added to an adenine base on an adjacent nucleotide. Plays a role in the binding of the ribosome to mRNA and affects mRNA stability and the removal of introns.

5′ end

End of a polynucleotide chain at which a phosphate group is attached to the $5'$-carbon atom of the sugar in the nucleotide.

5′ splice site

The $5'$ end of an intron where cleavage takes place in RNA splicing.

5′ untranslated region (5′ UTR)

Sequence of nucleotides at the $5'$ end of mRNA; does not encode the amino acids of a protein.

fixation

When one allele reaches a frequency of 1 in a population, at which point all individuals in the population are homozygous for one allele.

flanking direct repeat

Short, directly repeated sequence produced on either side of a transposable element when the element inserts into DNA.

forward genetics

Traditional approach to the study of gene function that begins with a mutant phenotype and proceeds to a gene that encodes the phenotype.

forward mutation

Mutation that alters a wild-type phenotype.

founder effect

Sampling error that results from the establishment of a population by a small number of individuals; leads to genetic drift.

fragile site

Constriction or gap that appears at a particular location on a chromosome when cells are cultured under special conditions.

fragile-X syndrome

A form of X-linked intellectual disability that appears primarily in males; associated with a fragile site that results from an expanding trinucleotide repeat.

frameshift mutation

Mutation that alters the reading frame of a gene.

frequency distribution

A concise graphical method of summarizing values. In genetics, the phenotypes found in a group of individuals are usually displayed as a frequency distribution. Typically, the phenotypic classes are plotted on the horizontal (*x*) axis, and the numbers (or proportions) of individuals in each class are plotted on the vertical (*y*) axis.

functional genomics

Area of genomics that studies the functions of the genetic information contained within genomes.

G_0 (gap 0)

Nondividing stage of the cell cycle.

G_1 (gap 1)

Stage of interphase in the cell cycle in which the cell grows and develops.

G_2 (gap 2)

Stage of interphase in the cell cycle that follows DNA replication. In G_2, the cell prepares for division.

gain-of-function mutation

Mutation that causes the cell to produce a protein or gene product whose function is not normally present; the result could be an entirely new gene product or one produced in an inappropriate tissue or at an inappropriate time in development.

gametic isolation

Reproductive isolation due to the incompatibility of gametes. Mating between members of different species may take place, but the gametes do not form zygotes. Seen in many plants in which pollen from one species cannot fertilize the ovules of another species.

gametophyte

Haploid phase of the life cycle in plants.

gap genes

In fruit flies, a set of segmentation genes that define large sections of the embryo. Mutations in gap genes usually eliminate whole groups of adjacent segments.

gel electrophoresis

Technique for separating charged molecules (such as proteins or nucleic acids) on the basis of molecular size or charge, or both.

gender

A sexual category assigned by an individual or others based on behavior and cultural practices.

gene

An inherited factor that helps determine a trait; often defined at the molecular level as a DNA sequence that is transcribed into an RNA molecule.

gene cloning

Insertion of DNA fragments into bacteria in such a way that the fragments will be stable and will be copied by the bacteria.

gene desert

A region of a genome that is gene poor—that is, a long stretch of DNA, possibly consisting of hundreds of thousands to millions of base pairs, that is completely devoid of any known genes or other functional sequences.

gene family

Set of genes that are similar in sequence and that arose through duplication events; often encode different protein products.

gene flow

See **migration**.

gene interaction

Interaction between genes at different loci that affect the same characteristic.

gene interaction variance

Component of genetic variance that can be attributed to gene interaction (interaction between genes at different loci).

gene mutation

Mutation that affects a single gene or locus.

gene pool

Set of all genes in a population.

generalized transduction

Transduction in which any gene may be transferred between bacteria by viruses.

general transcription factor

Protein that binds to a eukaryotic promoter near the transcription start site; part of the basal transcription apparatus that initiates transcription.

gene regulation

Mechanisms and systems that control the expression of genes.

gene therapy

The direct transfer of genes into human patients to treat disease.

genetic bottleneck

Sampling error that arises when a population undergoes a drastic reduction in size; leads to genetic drift.

genetic conflict hypothesis

Suggestion that genomic imprinting evolved because there are different and conflicting evolutionary pressures acting on maternal and paternal alleles for genes that affect fetal growth. For example, paternally derived alleles often favor maximum fetal growth, whereas maternally

derived alleles favor more limited fetal growth because of the high cost of fetal growth to the mother.

genetic correlation

Phenotypic correlation due to an effect of the same gene or genes on two or more characteristics.

genetic counseling

Educational process that helps patients and family members deal with many aspects of having a genetic condition.

genetic drift

Change in allelic frequencies due to a sampling error.

genetic engineering

See **recombinant DNA technology**.

genetic–environmental interaction variance

Component of phenotypic variance that results from an interaction between genotype and environment that causes genotypes to be expressed differently in different environments.

Genetic Information Nondiscrimination Act (GINA)

U.S. law prohibiting health insurers from using genetic information to make decisions about health-insurance coverage and rates; prevents employers from using genetic information in employment decisions, and prevents health insurers and employers from asking or requiring a person to take a genetic test.

genetic (linkage) map

Map of the relative distances between genetic loci, markers, or other chromosome regions determined by rates of recombination; measured in recombination frequencies or map units.

genetic marker

Any variable gene or DNA sequence used to identify a location on a genetic or physical map.

genetic maternal effect

The phenotype of the offspring is determined by the genotype of the mother.

genetic mosaicism

Condition in which regions of tissue within a single individual have different chromosome constitutions.

genetic rescue

Introduction of new genetic variation into an inbred population in an effort to improve the health of the population and better ensure its long-term survival.

genetics

The study of heredity; how inherited variation is encoded, replicated, and expressed; and how it evolves over time.

genetic variance

Component of phenotypic variance that is due to genetic differences among individual members of a population.

gene tree

Phylogenetic tree representing the evolutionary relationships among organisms or DNA sequences.

genic sex determination

Sex determination in which the sexual phenotype is specified by genotypes at one or more loci, but there are no obvious differences in the chromosomes of males and females.

genome

Complete set of genetic instructions for any organism.

genome-wide association study

Looks for nonrandom associations between the presence of a trait and alleles at many different loci scattered across a genome—that is, for associations between traits and particular suites of alleles in a population.

genomic imprinting

Differential expression of a gene depending on whether it is inherited from the male or female parent.

genomic library

Collection of bacterial colonies or phages containing DNA fragments that constitute the entire genome of an organism.

genomics

Study of the content, organization, function, and evolution of genetic information contained in whole genomes.

genotype

The set of alleles possessed by an individual organism.

genotypic frequency

Proportion of a particular genotype within a population.

germ-line mutation

Mutation in a germ-line cell (one that gives rise to gametes).

germ-plasm theory

Theory stating that cells in the reproductive organs carry a complete set of genetic information.

G_2/M checkpoint

Checkpoint in the cell cycle near the end of G_2; after this checkpoint has been passed, the cell undergoes mitosis.

G-rich $3'$ overhang

A guanine-rich sequence of nucleotides that protrudes beyond the complementary C-rich strand at the end of a chromosome.

group I introns

A class of introns which are capable of self-splicing; found in some genes of bacteria, bacteriophages, and eukaryotes.

group II introns

A class of introns which are capable of self-splicing; found in some protein-encoding genes in mitochondria, chloroplasts, archaea, and a few bacteria.

G_1/S checkpoint

Checkpoint in the cell cycle near the end of G_1; after this checkpoint has been passed, DNA replicates, and the cell is committed to dividing.

guanine (G)

Purine base in DNA and RNA.

guide RNA (gRNA)

RNA molecule that serves as a template for an alteration made in mRNA during RNA editing.

gynandromorph

Individual organism that is a genetic mosaic for the sex chromosomes, possessing tissues with different sex-chromosome constitutions.

gyrase

See **DNA gyrase**.

hairpin

Secondary structure formed when sequences of nucleotides on the same polynucleotide strand are complementary and pair with each other.

haploid

Possessing a single set of chromosomes (one genome).

haploinsufficiency

Appearance of a mutant phenotype in an individual cell or organism that is heterozygous for a normally recessive trait.

haploinsufficient gene

Gene that must be present in two copies for normal function. If one copy of the gene is missing, a mutant phenotype is produced.

haplotype

A specific set of linked genetic variants or alleles on a single chromosome or on part of a chromosome.

Hardy–Weinberg equilibrium

Frequencies of genotypes when the conditions of the Hardy–Weinberg law are met.

Hardy–Weinberg law

Principle of population genetics stating that if a pollution is large; randomly mating; and not affected by mutation, migration, or natural selection, then allelic frequencies of a population do not change and the genotypic frequencies stabilize after one generation in the proportions p^2 (the frequency of *AA*), $2pq$ (the frequency of *Aa*), and q^2 (the frequency of *aa*), where p equals the frequency of allele *A* and q equals the frequency of allele *a*.

H-DNA

DNA structure consisting of three nucleotide strands (triplex DNA); can occur when a single nucleotide strand from one part of a DNA molecule pairs with double-stranded DNA from another part of the molecule.

heat map

A visual (color) representation of a continuous variable, like the expression of a gene.

heat-shock protein

Protein produced by many cells in response to extreme heat and other stresses that helps the cells prevent damage from such stressing agents.

helicase

See **DNA helicase**.

hemizygosity

Possession of a single allele at a locus. Males of organisms with XX-XY sex determination are hemizygous for X-linked loci because their cells possess a single X chromosome.

heritability

Proportion of total phenotypic variation that is due to genetic differences. *See also* **broad-sense heritability**, **narrow-sense heritability**.

hermaphroditism

Condition in which an individual organism possesses both male and female reproductive structures. True hermaphrodites produce both male and female gametes.

heterochromatin

Chromatin that remains in a highly condensed state throughout the cell cycle; found at the centromeres and telomeres of most chromosomes.

heteroduplex DNA

DNA consisting of two strands, each of which is from a different chromosome.

heterogametic sex

The sex (male or female) that produces two types of gametes with respect to sex chromosomes. For example, in the XX-XY sex-determining system, the male produces both X-bearing and Y-bearing gametes.

heterokaryon

Cell possessing two nuclei derived from different cells through cell fusion.

heteroplasmy

Occurrence of two or more distinct variants of DNA within the cytoplasm of a single cell.

heterozygote advantage

See **overdominance**.

heterozygote screening

Testing of members of a population to identify heterozygous carriers of a disease-causing allele who are healthy but have the potential to produce children who have the disease.

heterozygous

Having two different alleles at a locus.

highly repetitive DNA
DNA that consists of short sequences that are present in hundreds of thousands to millions of copies that are repeated in tandem and are clustered in certain regions of chromosomes.
histone
Low-molecular-weight protein found in eukaryotes that associates closely with DNA to form chromosomes.
histone code
Modifications of histone proteins, such as the addition or removal of phosphate groups, methyl groups, or acetyl groups, that encode information affecting how genes are expressed.
Holliday intermediate
Structure that forms in homologous recombination; consists of two duplex molecules connected by a cross bridge.
Holliday junction
Special structure resulting from homologous recombination that is initiated by single-strand breaks in a DNA molecule.
holoenzyme
Complex of an enzyme and other protein factors necessary for its complete function.
homeobox
Conserved subset of nucleotides found in homeotic genes.
homeotic complex (HOM-C)
Major cluster of homeotic genes in fruit flies; consists of the *Antennapedia* complex, which affects the development of the adult fly's head and anterior segments, and the *bithorax* complex, which affects the adult fly's posterior thoracic and abdominal segments.
homeotic genes
Genes that determine the identity of individual segments or parts in an early embryo. Mutations in homeotic genes cause body parts to appear in the wrong places.
homogametic sex
The sex (male or female) that produces gametes that are all alike with respect to sex chromosomes. For example, in the XX-XY sex-determining system, the female produces only X-bearing gametes.
homologous genes
Evolutionarily related genes descended from a gene in a common ancestor.
homologous pair
A pair of chromosomes that are alike in structure and size and that carry genetic information for the same set of hereditary characteristics. One chromosome of a homologous pair is inherited from the male parent, and the other is inherited from the female parent.
homologous recombination
Exchange of genetic information between homologous DNA molecules.
homoplasmy

Presence of only one version of DNA within the cytoplasm of a single cell.

homozygous

Having two identical alleles at a locus.

horizontal gene transfer

Transfer of genes from one organism to another by a mechanism other than reproduction.

Hox gene

Gene that contains a homeobox.

human microbiome

The community of bacteria associated with humans.

human papillomavirus (HPV)

Virus associated with cervical cancer.

Human Proteome Project

Project with the goal of identifying and characterizing all proteins in the human body.

humoral immunity

Type of immunity resulting from antibodies produced by specialized lymphocytes called B cells.

hybrid breakdown

Reproductive isolating mechanism in which closely related species are capable of mating and producing viable and fertile F_1 progeny, but genes do not flow between the two species because further crossing of the hybrids produces inviable or sterile offspring.

hybrid dysgenesis

Sudden appearance of numerous mutations, chromosome aberrations, and sterility in the offspring of a cross between a male fruit fly that possesses *P* elements and a female fly that lacks them.

hybrid inviability

Reproductive isolating mechanism in which mating between two organisms of different species takes place and hybrid offspring are produced, but the resulting embryos are not viable.

hybridization

Pairing of two partly or fully complementary single-stranded polynucleotide chains.

hybrid sterility

Reproductive isolating mechanism in which hybrid embryos complete development but are sterile; exemplified by mating between donkeys and horses to produce a mule, a viable but usually sterile offspring.

hypostatic gene

Gene that is masked or suppressed by the action of a gene at a different locus.

identical twins

See **monozygotic twins**.

inbreeding

A form of nonrandom mating; preferential mating between related individuals.

inbreeding coefficient

A measure of inbreeding; the probability (ranging from 0 to 1) that two alleles are identical by descent.

inbreeding depression

Decreased fitness arising from inbreeding; often due to the increased appearance of lethal or deleterious traits with inbreeding.

incomplete dominance

Type of dominance in which the phenotype of the heterozygote falls in between the phenotypes of the two homozygotes.

incomplete linkage

Linkage between genes that exhibit some crossing over; intermediate in its effects between independent assortment and complete linkage.

incomplete penetrance

The genotype does not always produce the expected phenotype.

incorporated error

Incorporation of a damaged nucleotide or mismatched base pair into a DNA molecule.

independent assortment

See **principle of independent assortment**.

induced mutation

Mutation that results from environmental agents, such as chemicals or radiation.

induced pluripotent stem cells (iPSCs)

Somatic cells that have been artificially induced to dedifferentiate and revert to pluripotent stem cells capable of becoming many types of cells.

inducer

Substance that stimulates transcription in an inducible system of gene regulation; usually a small molecule that binds to a repressor protein and alters that repressor so that it can no longer bind to DNA and inhibit transcription.

inducible operon

Operon in which transcription is normally off (not taking place); something must happen to induce transcription, or turn it on.

in-frame deletion

Deletion of some multiple of three nucleotides that do not alter the reading frame of the gene.

in-frame insertion

Insertion of some multiple of three nucleotides that do not alter the reading frame of the gene.

inheritance of acquired characteristics

Early notion of inheritance proposing that acquired traits are passed on to offspring.

initiation codon

The first codon of the mRNA to specify an amino acid (fMet in bacterial cells; Met in eukaryotic cells); most often AUG.

initiation factor 1 (IF-1)

Protein required for the initiation of translation in bacterial cells; enhances the dissociation of the large and small ribosomal subunits.

initiation factor 2 (IF-2)

Protein required for the initiation of translation in bacterial cells; forms a complex with GTP and the charged initiator tRNA and then delivers the charged tRNA to the initiation complex.

initiation factor 3 (IF-3)

Protein required for the initiation of translation in bacterial cells; binds to the small ribosomal subunit and prevents the large subunit from binding during initiation.

initiator protein

Protein that binds to an origin of replication and causes a short section of DNA to unwind, allowing helicase and other single-strand-binding proteins to attach to the polynucleotide strand.

insertion

Mutation in which one or more nucleotide pairs are added to a DNA sequence.

insertion sequence (IS)

Simplest type of transposable element in bacteria and plasmids; carries only the genetic information necessary for its movement.

in situ hybridization

Method used to determine the chromosomal location of a gene or another specific DNA fragment or the tissue distribution of an mRNA by using a labeled probe that is complementary to the sequence of interest.

insulator

DNA sequence that blocks the effect of an enhancer in a position-dependent manner; must be located between the enhancer and the promoter to have blocking activity; may also limit the spread of changes in chromatin structure.

integrase

Enzyme that inserts prophage, or proviral, DNA into a host chromosome.

interactome

Complete set of protein interactions in a cell.

intercalating agent

Molecule that is about the same size as a nucleotide and may become sandwiched between adjacent bases in DNA, distorting the three-dimensional structure of the DNA helix and causing single-nucleotide insertions and deletions in replication.

interference

Degree to which one crossover interferes with additional crossovers in the same region.

intergenic suppressor mutation

Suppressor mutation that occurs in a gene (locus) other than the one bearing the original mutation that it suppresses.

interkinesis

Period between meiosis I and meiosis II.

internal promoter
Promoter located within the sequences of DNA that are transcribed into RNA.
interphase
Major phase of the cell cycle between cell divisions. In interphase, the cell grows, develops, and functions.
interspersed repeats
Repeated sequences that are scattered throughout the genome.
intragenic suppressor mutation
Suppressor mutation that occurs in the same gene (locus) as the mutation that it suppresses.
intron
Noncoding sequence between coding regions in a eukaryotic gene; removed from the RNA after transcription.
inverted repeats
Sequences on the same strand that are inverted and complementary.
isoaccepting tRNAs
Different tRNAs that accept the same amino acid but have different anticodons.
isotopes
Different forms of an element that have the same number of protons and electrons but differ in the number of neutrons in the nucleus.
junctional diversity
Addition or deletion of nucleotides at the junctions of gene segments brought together in the somatic recombination of genes that encode antibodies and T-cell receptors.
karyotype
The complete set of chromosomes possessed by an organism; usually presented as a picture of a complete set of its metaphase chromosomes.
kinetochore
Set of proteins that assemble on the centromere, providing the point of attachment for spindle microtubules.
Klinefelter syndrome
Human condition in which cells contain one or more Y chromosomes and multiple X chromosomes (most commonly XXY but may also be XXXY, XXXXY, or XXYY). People with Klinefelter syndrome are male in appearance but frequently have small testes and reduced facial and pubic hair; they are often taller than normal and sterile, and most have normal intelligence.
knock-in mouse
Mouse that carries a foreign DNA sequence inserted at a specific chromosome location.
knockout mouse
Mouse in which a normal gene has been fully disabled (knocked out).
lagging strand
DNA strand that is replicated discontinuously.

large ribosomal subunit
The larger of the two subunits of a functional ribosome.
lariat
Loop-like structure created in the splicing of nuclear pre-mRNA when the $5'$ end of an intron is attached to the branch point.
leading strand
DNA strand that is replicated continuously.
leptotene
First substage of prophase I in meiosis. In leptotene, chromosomes contract and become visible.
lethal allele
Allele that causes death at an early stage in development—often before birth—so that some genotypes do not appear among the progeny. A recessive lethal allele kills individuals that are homozygous for the allele; a dominant lethal allele kills both heterozygotes and homozygotes.
lethal mutation
Mutation that causes premature death.
LINE
See **long interspersed element**.
linkage analysis
Gene mapping based on the detection of physical linkage between genes, as measured by the rate of recombination in the progeny of a cross.
linkage disequilibrium
Nonrandom association between alleles in a haplotype.
linkage group
A group of linked genes.
linkage map
See **genetic map**.
linked genes
Genes located close together on the same chromosome.
linker
Small synthetic DNA fragment that contains one or more restriction sites; can be attached to the ends of any piece of DNA and used to insert it into a vector.
linker DNA
Stretch of DNA separating two nucleosomes.
locus (pl., loci)
Position on a chromosome where a specific gene is located.
lod (logarithm of odds) score
Logarithm of the ratio of the probability of obtaining a set of observations, assuming a specified degree of linkage, to the probability of obtaining the same set of observations with independent assortment; used to assess the likelihood of linkage between genes from pedigree data.

long interspersed element (LINE)
Long DNA sequence repeated many times and interspersed throughout the genome.
long noncoding RNA (lncRNA)
A class of relatively long RNA molecules found in eukaryotes that do not code for proteins; they provide a variety of functions, including regulation of gene expression.
loss-of-function mutation
Mutation that causes the complete or partial absence of normal function.
loss of heterozygosity
Inactivation or loss of the wild-type allele of a heterozygote.
Lyon hypothesis
Proposal by Mary Lyon in 1961 that one X chromosome in each female cell becomes inactivated (a Barr body), and that which of the X chromosomes is inactivated is random and varies from cell to cell.
lysogenic cycle
Life cycle of a bacteriophage in which phage genes first integrate into the bacterial chromosome and are not immediately transcribed and translated.
lytic cycle
Life cycle of a bacteriophage in which phage genes are transcribed and translated, new phage particles are produced, and the host cell is lysed.
major histocompatibility complex (MHC) antigens
A large and diverse group of antigens found on the surfaces of cells that mark those cells as self; encoded by a large cluster of genes known as the major histocompatibility complex. T cells simultaneously bind to foreign and MHC antigens.
malignant
Tumor consisting of cells that invade other tissues.
map-based sequencing
Method of sequencing a genome in which short sequenced fragments are assembled into the correct sequence in contigs with the use of genetic or physical maps.
mapping function
Mathematical function that relates recombination frequencies to actual physical distances between genes.
map unit (m.u.)
Unit of measure for distances on a genetic map; also called a **centiMorgan**. One map unit equals a 1% recombination frequency.
mass spectrometry
Method for precisely determining the molecular mass of a molecule by using the migration rate of an ionized molecule in an electrical field.
maternal blood screening test

Method of screening for genetic conditions in a fetus by examining levels of certain substances in the blood of the mother. For example, the level of α-fetoprotein in maternal blood provides information about the probability that a fetus has a neural-tube defect.

mean

Statistic that provides information about the center of a distribution of measurements; calculated by adding all the individual measurements and dividing by the total number of measurements; also called the average.

mechanical isolation

Reproductive isolation resulting from anatomical differences that prevent successful exchange of gametes.

mediator

Complex of proteins that is one of the components of the basal transcription apparatus.

megaspore

One of the four products of meiosis in plants.

megasporocyte

Diploid reproductive cell in the ovary of a plant that undergoes meiosis to produce haploid megaspores.

meiosis

Process by which the chromosomes of a eukaryotic cell divide to give rise to haploid reproductive cells. Consists of two divisions: meiosis I and meiosis II.

meiosis I

First phase of meiosis. In meiosis I, chromosome number is reduced by half.

meiosis II

Second phase of meiosis. Events in meiosis II are similar to those in mitosis.

melting temperature

The temperature at which DNA denatures.

memory cell

Long-lived lymphocyte that is among the clone of cells generated when a foreign antigen is encountered. If the same antigen is encountered again, the memory cells quickly divide and give rise to another clone of cells specific for that particular antigen.

Mendelian population

A group of interbreeding, sexually reproducing individuals that have a common set of genes.

meristic characteristic

Characteristic whose phenotype varies in whole numbers, such as number of vertebrae, but may be caused by continuous genetic variation.

merozygote

See **partial diploid**.

messenger RNA (mRNA)

RNA molecule that carries the coding instructions for a polypeptide chain from DNA to a ribosome.

metacentric chromosome
Chromosome in which the two chromosome arms are approximately the same length.
metagenomics
A field of genetics in which the genome sequences of an entire group of organisms that inhabit a common environment are sampled and determined.
metaphase
Stage of mitosis in which chromosomes align in the center of the cell.
metaphase I
Stage of meiosis I in which homologous pairs of chromosomes align in the center of the cell along the metaphase plate.
metaphase II
Stage of meiosis II in which individual chromosomes align in the center of the cell.
metaphase plate
Plane in a cell between two spindle poles. In metaphase, chromosomes align on the metaphase plate.
metastasis
The movement of cells that separate from malignant tumors to other sites, where they establish secondary tumors.
microarray
Ordered array of known DNA fragments, fixed to a solid support in an orderly pattern, which serve as probes to detect the presence of complementary sequences; often used to assess the expression of genes in various tissues and under different conditions.
microbiome
Complete set of all bacteria found in a particular environment; for example, the human microbiome consists of all bacteria found in and on the human body.
microRNA (miRNA)
Small RNA molecules, typically 21 or 22 bp in length, produced by cleavage of double-stranded RNA arising from small hairpins within RNA that is mostly single stranded. The miRNAs combine with proteins to form a complex that binds (imperfectly) to mRNA molecules and inhibits their translation.
microsatellite
Very short DNA sequence repeated in tandem; also called a **short tandem repeat (STR)**.
microspore
Haploid product of meiosis in plants.
microsporocyte
Diploid reproductive cell in the stamen of a plant; undergoes meiosis to produce four haploid microspores.
microtubule

Long fiber composed of the protein tubulin; plays an important role in the movement of chromosomes in mitosis and meiosis.

migration

Movement of genes from one population to another; also called **gene flow**.

minimal medium

Medium that contains the nutrients required by only prototrophic bacteria—typically a carbon source, essential elements such as nitrogen and phosphorus, certain vitamins, and other required ions and nutrients.

mismatch repair

Process that corrects mismatched nucleotides in DNA after replication has been completed. Enzymes excise incorrectly paired nucleotides from the newly synthesized strand and use the original nucleotide strand as a template for replacing them.

missense mutation

Mutation in which a base substitution results in a different amino acid in the protein encoded.

mitochondrial DNA (mtDNA)

DNA in mitochondria; has some characteristics in common with bacterial DNA and typically consists of a circular molecule that lacks histone proteins and encodes some of the rRNAs, tRNAs, and proteins found in mitochondria.

mitochondrial replacement therapy

Methods that combine the nuclear DNA of a female who carries a mitochondrial mutation with that of a sperm and the egg cytoplasm of a healthy donor, creating a “three-parent baby.”

mitosis

Process by which the nucleus of a eukaryotic cell divides.

mitosis-promoting factor (MPF)

Protein functioning in the control of the cell cycle; consists of a cyclin combined with cyclin-dependent kinase (CDK). Active MPF stimulates mitosis.

mitotic spindle

Array of microtubules that radiate from two poles; moves chromosomes in mitosis and meiosis.

model genetic organism

An organism that is widely used in genetic research because it has characteristics, such as short generation time and large numbers of progeny, that make it particularly useful for genetic analysis.

moderately repetitive DNA

A major class of repetitive DNA, which typically consists of sequences from 150 to 300 bp in length that are repeated thousands of times.

modified base

Any of several rare bases found in some RNA molecules. Such bases are modified forms of the standard bases (adenine, cytosine, guanine, and uracil).

molecular chaperone

Molecule that assists in the proper folding of another molecule.

molecular clock

Use of molecular differences to estimate the time of evolutionary divergence between organisms; assumes a roughly constant rate at which one neutral mutation replaces another.

molecular genetics

Study of the chemical nature of genetic information and how it is encoded, replicated, and expressed.

molecular motor

Specialized protein that moves cellular components.

monoecious

Refers to an individual organism that has both male and female reproductive structures.

monohybrid cross

Cross between two individuals that differ in a single characteristic—more specifically, a cross between individuals that are homozygous for different alleles at the same locus (*AA* × *aa*); also refers to a cross between two individuals that are heterozygous for two alleles at a single locus (*Aa* × *Aa*).

monosomy

Absence of one of the chromosomes of a homologous pair.

monozygotic twins

Twins that arise when a single egg, fertilized by a single sperm, splits early in development into two separate embryos; also called identical twins.

morphogen

Molecule whose concentration gradient affects the developmental fate of surrounding cells.

M (mitotic) phase

The major phase of the cell cycle that encompasses active cell division; includes mitosis (nuclear division) and cytokinesis (cytoplasmic division).

mRNA surveillance

Mechanisms for the detection and elimination of mRNAs that contain errors that may create problems in the course of translation.

multifactorial characteristic

Characteristic determined by multiple genes and environmental factors.

multigene family

Set of genes similar in sequence that arose through repeated duplication events and often encode different protein products.

multiple alleles

Presence of more than two alleles within a group of organisms; however, each individual member of the group has only two of the possible alleles.

multiple 3′ cleavage sites

The presence of more than one 3′ cleavage site on a single pre-mRNA, which allows cleavage and polyadenylation to take place at different sites, producing mRNAs of different lengths.

multiplication rule

Rule stating that the probability of two or more independent events taking place together is calculated by multiplying their probabilities.

mutagen

Any environmental agent that significantly increases the rate of mutation above the spontaneous rate.

mutagenesis screen

Method for identifying genes that influence a specific phenotype. Random mutations are induced in a population of organisms, and individual organisms with mutant phenotypes are identified. These individual organisms are crossed to determine the genetic basis of the phenotype and to map the location of mutations that cause the phenotype.

mutation

Heritable change in genetic information.

mutation rate

Frequency with which a wild-type allele changes to a mutant allele; generally expressed as the number of mutations per biological unit (that is, mutations per cell division, per gamete, or per round of replication).

narrow-sense heritability

Proportion of phenotypic variance that results from the additive genetic variance.

natural selection

Differential reproduction of individuals with different genotypes.

negative assortative mating

A tendency for unlike individuals to mate.

negative control

Transcriptional control in which a regulatory protein is a repressor, binding to DNA and inhibiting transcription.

negative supercoiling

Tertiary structure that forms when strain is placed on a DNA helix by underrotating.

neutral mutation

Mutation that changes the amino acid sequence of a protein but does not alter the function of the protein.

neutral-mutation hypothesis

Proposal that much of the molecular variation seen in natural populations is adaptively neutral and unaffected by natural selection; that is, that individuals with different molecular variants have equal fitness.

newborn screening

Testing for genetic disorders in newborn infants.

next-generation sequencing technologies
Sequencing methods that are capable of simultaneously determining the sequences of many DNA fragments; these technologies are much faster and less expensive than the Sanger dideoxy sequencing method.
nitrogenous base
Nitrogen-containing base that is one of the three parts of a nucleotide.
node
Point in a phylogenetic tree where branches split; represents a common ancestor.
no-go decay (NGD)
An mRNA surveillance system in eukaryotes that helps remove stalled ribosomes resulting from secondary structures in mRNA, chemical damage to mRNA, premature stop codons, and ribosomal defects.
noncomposite transposon
Type of transposable element in bacteria that lacks insertion sequences, possesses a gene for transposase, and has terminal inverted repeats.
nondisjunction
Failure of homologous chromosomes or sister chromatids to separate in meiosis or mitosis.
nonhistone chromosomal protein
One of a heterogeneous assortment of nonhistone proteins in chromatin.
noninvasive prenatal genetic diagnosis
Genetic test performed on a fetus without taking a tissue sample from the fetus; usually performed by testing fetal DNA found within the maternal blood.
nonoverlapping genetic code
Refers to the fact that, generally, each nucleotide is part of only one codon and encodes only one amino acid in a protein.
nonreciprocal translocation
Movement of a chromosome segment to a nonhomologous chromosome or chromosomal region without any (or with unequal) reciprocal exchange of segments.
nonrecombinant (parental) gamete
Gamete that contains only the original combinations of alleles that were present in the parents.
nonrecombinant (parental) progeny
Progeny that possess only the original combinations of alleles that were present in the P generation.
nonreplicative transposition
Type of transposition in which a transposable element excises from an old site and moves to a new site, resulting in no net increase in the number of copies of the transposable element.
nonsense codon
See **stop codon**.
nonsense-mediated mRNA decay (NMD)

Process that results in the rapid elimination of mRNA containing a premature termination codon.

nonsense mutation

Mutation that changes a sense codon (one that specifies an amino acid) into a stop codon (one that terminates translation).

nonstop mRNA decay

Mechanism in eukaryotic cells for dealing with ribosomes stalled at the 3′ end of an mRNA that lacks a termination codon, in which a protein binds to the A site of the stalled ribosome and recruits other proteins that degrade the mRNA from the 3′ end.

nontemplate strand

The DNA strand that is complementary to the template strand; not ordinarily used as a template during transcription.

normal distribution

Common type of frequency distribution that exhibits a symmetrical, bell-shaped curve; usually arises when a large number of independent factors contribute to a measured value.

Northern blotting

Process by which RNA is transferred from a gel to a solid support, such as a nitrocellulose or nylon membrane.

nuclear envelope

Membrane that surrounds the genetic material in eukaryotic cells to form a nucleus; segregates the DNA from other cellular contents.

nuclear matrix

Network of protein fibers in the nucleus; holds the nuclear contents in place.

nuclear pre-mRNA introns

A class of introns in nuclear protein-encoding genes that are removed by spliceosome-mediated splicing.

nucleoid

Bacterial DNA confined to a definite region of the cytoplasm.

nucleoside

Ribose or deoxyribose bonded to a nitrogenous base.

nucleosome

Basic repeating unit of chromatin, consisting of a core of eight histone proteins (two each of H2A, H2B, H3, and H4) and about 146 bp of DNA that wraps around the core about two times.

nucleotide

Repeating unit of DNA or RNA made up of a sugar, a phosphate, and a nitrogenous base.

nucleotide-excision repair

DNA repair that removes bulky DNA lesions and other types of DNA damage.

nucleus

Compartment in eukaryotic cells that is enclosed by the nuclear membrane and contains the genetic material.

nullisomy

Absence of both members of a homologous pair of chromosomes ($2n - 2$).

Okazaki fragment

Short length of newly synthesized DNA produced by discontinuous replication on the lagging strand; these fragments are eventually joined together.

oligonucleotide-directed mutagenesis

Method of site-directed mutagenesis that uses an oligonucleotide to introduce a mutant sequence into a DNA molecule.

oncogene

Dominant-acting gene that stimulates cell division, leading to the formation of tumors and contributing to cancer; arises from a mutated copy of a normal cellular gene (proto-oncogene).

one gene, one enzyme hypothesis

Proposal by Beadle and Tatum that genes function by encoding enzymes, and each gene encodes a separate enzyme.

one gene, one polypeptide hypothesis

Modification of the one gene, one enzyme hypothesis; proposes that each gene encodes a separate polypeptide chain.

oogenesis

The production of gametes in a female animal.

oogonium

Diploid cell in the ovary; capable of undergoing meiosis to produce an egg cell.

open reading frame (ORF)

Continuous sequence of DNA nucleotides that contains a start codon and a stop codon in the same reading frame; is assumed to be a gene that encodes a protein, but in many cases, the protein has not yet been identified.

operator

DNA sequence in an operon to which a regulator protein binds; this binding affects the rate of transcription of the structural genes.

operon

A group of bacterial structural genes that are transcribed together, along with their promoter and additional sequences that control their transcription.

origin of replication

Site where DNA replication is initiated.

origin-recognition complex (ORC)

Multiprotein complex that binds to an origin of replication and unwinds the DNA around it to initiate DNA replication.

orthologs

Homologous genes found in different species that evolved from the same gene in a common ancestor.

outcrossing

Preferential mating between unrelated individuals.

overdominance

Selection in which the heterozygote has higher fitness than either homozygote; also called **heterozygote advantage**.

ovum

The mature female gamete; the larger cell, which acquires most of the cytoplasm during oogenesis.

pachytene

Third substage of prophase I in meiosis. The synaptonemal complex forms during pachytene.

pair-rule genes

Set of segmentation genes in fruit flies that define regional sections of the embryo and affect alternate segments. Mutations in these genes often cause the deletion of every other segment.

palindrome

Sequence of nucleotides that reads the same on complementary strands; inverted repeats.

pangenesis

Early concept of heredity proposing that particles carry genetic information from different parts of the body to the reproductive organs.

paracentric inversion

Chromosome inversion that does not include the centromere in the inverted region.

paralogs

Homologous genes in the same species that arose through the duplication of a single ancestral gene.

paramutation

Epigenetic change in which one allele of a genotype alters the expression of another allele; the altered expression persists for several generations, even after the altering allele is no longer present.

parental gamete

See **nonrecombinant gamete**.

parental progeny

See **nonrecombinant progeny**.

partial diploid

Bacterial cell that possesses two copies of some genes, one on the bacterial chromosome and the other on an extra piece of DNA (usually a plasmid); also called a merozygote.

passenger

Mutation that arises randomly in the process of tumor development and does not contribute to cancer progression.

Patau syndrome (trisomy 13)
Human condition characterized by severe intellectual disability, small head, sloping forehead, small eyes, cleft lip and palate, extra fingers and toes, and other disabilities; results from the presence of three copies of chromosome 13.

pedigree
Pictorial representation of a family history, essentially a family tree that outlines the inheritance of one or more characteristics.

penetrance
Percentage of individual organisms having a particular genotype that express the expected phenotype.

peptide bond
Chemical bond that connects amino acids in a protein.

peptidyl (P) site
One of the three sites in a ribosome occupied by a tRNA during translation. In the elongation stage, tRNAs move from the aminoacyl (A) site into the P site.

pericentric inversion
Chromosome inversion that includes the centromere in the inverted region.

P (parental) generation
The first generation in a genetic cross.

phage
See **bacteriophage**.

phenocopy
Phenotype produced by environmental factors alone that is the same as the phenotype produced by a genotype.

phenotype
Appearance or manifestation of a characteristic.

phenotypic correlation
Correlation between two or more phenotypes in the same individual.

phenotypic variance
Measure of the degree of phenotypic difference among a group of individuals; composed of genetic, environmental, and genetic–environmental interaction variances.

phosphate group
A phosphorus atom bonded to four oxygen atoms; one of the three components of a nucleotide.

phosphodiester linkage
A strong covalent bond that joins the $5'$-phosphate group of one nucleotide to the $3'$-hydroxyl group of the next nucleotide in a polynucleotide strand.

phylogenetic tree
Graphical representation of the evolutionary relationships among different organisms.

phylogeny

Evolutionary relationships among a group of organisms.

physical map

Map of physical distances between loci, genetic markers, or other chromosome segments; measured in base pairs.

pilus (pl., pili)

Extension of the cell membrane of some bacteria that allows conjugation to take place. When a pilus on one cell makes contact with a receptor on another cell, the pilus contracts and pulls the two cells together.

Piwi-interacting RNA (piRNA)

Small RNA molecule belonging to a class named after Piwi proteins, with which these molecules interact; similar to microRNA and small interfering RNA, has a role in suppressing the expression of transposable elements in reproductive cells.

plaque

Clear patch of lysed cells on a continuous layer of bacteria on the agar surface of a petri plate; each plaque represents a single original phage that multiplied and lysed many cells.

plasmid

Small, usually circular DNA molecule that is distinct from the bacterial chromosome; capable of replicating independently of the bacterial chromosome.

pleiotropy

Ability of one gene to affect multiple characteristics.

pluripotency

The property of being undifferentiated, with the capacity to form every type of cell in an organism.

poly(A)-binding protein (PABP)

Protein that binds to the poly(A) tail of eukaryotic mRNA and makes the mRNA more stable. There are several types of PABP.

poly(A) tail

String of adenine nucleotides added to the $3'$ end of a eukaryotic mRNA after transcription.

polycistronic mRNA

Single RNA molecule transcribed from a group of several genes; uncommon in eukaryotes.

polygenic characteristic

Characteristic encoded by genes at many loci.

polymerase chain reaction (PCR)

Method of enzymatically amplifying DNA fragments.

polynucleotide strand

Series of nucleotides linked together by phosphodiester bonds.

polypeptide

Chain of amino acids linked by peptide bonds; also called a protein.

polyploidy

Possession of more than two sets of chromosomes.

polyribosome
Messenger RNA molecule with several ribosomes attached to it.
polytene chromosome
Giant chromosome found in certain tissues of *Drosophila melanogaster*. Each polytene chromosome consists of a number of DNA molecules lying side by side.
population
(1) In statistics, the group of interest; often represented by a subset called a sample. (2) A group of members of the same species.
population genetics
Study of the genetic composition of populations and how that composition changes geographically and with the passage of time.
positional cloning
Method that allows for the isolation and identification of a gene by examining the inheritance of a phenotype in association with the inheritance of previously mapped genetic markers.
position effect
Dependence of the expression of a gene on the gene's location in the genome.
positive assortative mating
A tendency for like individuals to mate.
positive control
Transcriptional control in which a regulatory protein is an activator, stimulating transcription.
positive supercoiling
Tertiary structure that forms when strain is placed on a DNA helix by overrotating.
posttranslational modification
Alteration of a protein after translation; may include cleavage from a larger precursor protein, the removal of amino acids, and the attachment of other molecules to the protein.
postzygotic reproductive isolating mechanism
Reproductive isolating mechanism that operates after gametes from two different species have fused to form a zygote, either because the resulting hybrids are inviable or sterile or because reproduction breaks down in subsequent generations.
preformationism
Early concept of inheritance proposing that a miniature adult (*homunculus*) resides in either the egg or the sperm and increases in size in development, and that all traits are inherited from the parent that contributes the homunculus.
preimplantation genetic diagnosis (PGD)
Genetic testing on an embryo combined with in vitro fertilization to allow the implantation of embryos that are free of a specific genetic defect.
pre-messenger RNA (pre-mRNA)
The immediate products of transcription in eukaryotic cells. Modified after transcription to become mRNA.

presymptomatic genetic testing
Testing of healthy adults for genes that might predispose them to a genetic condition in the future.
prezygotic reproductive isolating mechanism
Reproductive isolating mechanism that prevents gametes from two different species from fusing and forming a hybrid zygote.
primary Down syndrome
Human condition caused by the presence of three copies of chromosome 21.
primary immune response
Initial clone of cells specific for a particular antigen and generated when the antigen is first encountered by the immune system.
primary oocyte
Oogonium that has entered prophase I.
primary spermatocyte
Spermatogonium that has entered prophase I.
primary structure of a protein
The amino acid sequence of a protein.
primase
Enzyme that synthesizes a short stretch of RNA on a DNA template; functions in replication to provide a $3'$-OH group for the attachment of a DNA nucleotide.
primer
Short stretch of RNA on a DNA template; provides a $3'$-OH group for the attachment of a DNA nucleotide at the initiation of replication.
principle of independent assortment (Mendel's second law)
Principle of heredity discovered by Mendel that states that genes encoding different characteristics (genes at different loci) separate independently; applies to genes located only on different chromosomes or to genes far apart on the same chromosome.
principle of segregation (Mendel's first law)
Principle of heredity discovered by Mendel that states that each individual diploid organism possesses two alleles at a locus and that these two alleles separate when gametes are formed, one allele going into each gamete.
probability
Likelihood of the occurrence of a particular event; more formally, the number of times that a particular event takes place, divided by the number of all possible outcomes. Probability values range from 0 to 1.
proband
The person from whom the pedigree is initiated.
probe

A DNA or RNA molecule with a base sequence that is complementary to a sequence of interest and will pair with it; used to find specific DNA sequences.

prokaryote

Unicellular organism with a relatively simple cell structure. Prokaryotes include bacteria (eubacteria) and archaea.

prometaphase

Stage of mitosis in which the nuclear membrane breaks down and the spindle microtubules attach to the chromosomes.

promoter

DNA sequence that the transcription apparatus recognizes and binds so as to initiate transcription; indicates the direction of transcription, which of the two DNA strands is to be read as the template, and the starting point of transcription.

proofreading

Process by which DNA polymerases remove and replace incorrectly paired nucleotides in the course of replication.

prophage

Phage genome that is integrated into a bacterial chromosome.

prophase

Stage of mitosis in which the chromosomes contract and become visible, the cytoskeleton breaks down, and the mitotic spindle begins to form.

prophase I

Stage of meiosis I in which chromosomes condense and pair, crossing over takes place, the nuclear membrane breaks down, and the mitotic spindle forms.

prophase II

Stage of meiosis in which events of interkinesis are reversed; chromosomes recondense, the spindle re-forms, and the nuclear membrane breaks down. Some cells skip this stage.

protein-coding region

The part of mRNA consisting of the nucleotides that specify the amino acid sequence of a protein.

protein domain

Region of a protein that has a specific shape or function.

protein kinase

Enzyme that adds phosphate groups to other proteins.

protein microarray

Large number of different proteins applied to a solid support in an orderly array of spots, each spot containing a different protein; used to analyze protein–protein interactions.

proteome

Set of all proteins found in a cell.

proteomics

Study of the proteome.

proto-oncogene
Normal cellular gene responsible for basic cellular function, but when mutated, it may become an oncogene and contribute to the development of cancer.
protospacer-adjacent motif (PAM)
Adjacent DNA sequence required for binding by a CRISPR-Cas effector complex; the complex associates with the PAM and unwinds the DNA nearby.
prototrophic
Capable of using a carbon source, essential elements such as nitrogen and phosphorus, certain vitamins, and other required ions and nutrients to synthesize all the compounds needed for growth and reproduction; a prototrophic bacterium can grow on a minimal medium.
provirus
A viral genome incorporated into a host chromosome.
pseudoautosomal region
Small region of the X and Y chromosomes that contains homologous gene sequences.
pseudodominance
Expression of a normally recessive allele due to a deletion on the homologous chromosome.
Punnett square
Shorthand method of determining the outcome of a genetic cross. On a grid, the gametes of one parent are written along the upper edge, and the gametes of the other parent are written down the left side. Within the cells of the grid, the alleles in the gametes are combined to form the genotype of the progency.
purine
Type of nitrogenous base in DNA and RNA. Adenine and guanine are purines.
pyrimidine
Type of nitrogenous base in DNA and RNA. Cytosine, thymine, and uracil are pyrimidines.
pyrimidine dimer
Structure in which a bond forms between two adjacent pyrimidine molecules on the same strand of DNA; distorts the normal configuration of the DNA molecule and often blocks replication.
quantitative characteristic
Continuous characteristic; displays a large number of possible phenotypes or is encoded by multiple genetic factors.
quantitative genetics
Genetic analysis of complex characteristics.
quantitative trait locus (QTL)
A chromosomal region containing genes that control polygenic characteristics.
quaternary structure of a protein
Interaction of two or more polypeptides to form a functional protein.
reading frame

Particular way in which a nucleotide sequence is read in groups of three nucleotides (codons) in translation; begins with a start codon and ends with a stop codon.

realized heritability

Heritability determined by a response-to-selection experiment.

real-time PCR

Modification of the polymerase chain reaction that is used to measure the starting amount of nucleic acid; the amount of DNA amplified is measured as the reaction proceeds.

reannealing

See **renaturation**.

recessive

Refers to an allele or a phenotype that is expressed only in homozygotes (*aa*); the recessive allele is not expressed in a heterozygote (*Aa*) phenotype.

reciprocal crosses

Pair of crosses in which the phenotypes of the male and female parents are reversed. For example, in one cross, a tall male is crossed with a short female, and in the other cross, a short male is crossed with a tall female.

reciprocal translocation

Reciprocal exchange of segments between two nonhomologous chromosomes.

recombinant DNA technology

Set of molecular techniques for locating, isolating, altering, combining, and studying DNA segments; also commonly called **genetic engineering**.

recombinant gamete

Gamete with new combinations of alleles.

recombinant progeny

Progeny with new combinations of alleles formed from recombinant gametes.

recombination

Process that produces new combinations of alleles.

recombination frequency

Proportion of recombinant progeny produced in a cross.

recursive splicing

A variation of splicing in which some long introns are removed in multiple steps.

regression

Analysis of how one variable changes in response to another variable.

regression coefficient

Statistic that measures how much one variable changes, on average, with a unit change in another variable.

regulator gene

Gene associated with an operon in bacterial cells that encodes a protein or RNA molecule that functions in controlling the transcription of other genes.

regulator protein
Protein produced by a regulator gene that binds to another DNA sequence and controls the transcription of other genes.
regulatory element
DNA sequence that affects the transcription of other DNA sequences to which it is physically linked.
regulatory gene
DNA sequence that encodes a protein or RNA molecule that interacts with other DNA sequences and affects transcription or translation of those sequences.
regulatory promoter
DNA sequence located immediately upstream of the eukaryotic core promoter; contains consensus sequences to which transcription factors bind.
relaxed state of DNA
Energy state of a DNA molecule when there is no structural strain on the molecule.
release factor
Protein required for the termination of translation; binds to a ribosome when a stop codon is reached and stimulates the release of the polypeptide chain, the tRNA, and the mRNA from the ribosome. Eukaryotic cells require two release factors (eRF-1 and eRF-2), whereas *E. coli* requires three (RF-1, RF-2, and RF-3).
renaturation
Process by which two complementary single-stranded DNA molecules pair; also called **reannealing**.
repetitive DNA
DNA sequences that exist in multiple copies in a genome.
replicated error
An incorporated error that is replicated, leading to a permanent mutation.
replication
Process by which DNA is synthesized from a single-stranded nucleotide template.
replication bubble
Segment of a DNA molecule that is unwinding and undergoing replication.
replication fork
Point at which a double-stranded DNA molecule separates into two single strands that serve as templates for replication.
replication licensing factor
Protein that ensures that replication takes place only once at each origin of replication; required at the origin before replication can be initiated and removed after the DNA has been replicated.
replication origin
See **origin of replication**.
replicative segregation

Random segregation of organelles into progeny cells in cell division. If two or more versions of an organelle are present in the parent cell, chance determines the proportion of each type that will segregate into each progeny cell.

replicative transposition

Type of transposition in which a copy of a transposable element moves to a new site while the original copy remains at the old site; increases the number of copies of the transposable element.

replicon

Unit of replication consisting of DNA from the origin of replication to the point at which replication on either side of the origin ends.

repressible operon

Operon in which transcription is normally on (taking place); something must happen to repress transcription, or turn it off.

repressor

Regulatory protein that binds to a DNA sequence and inhibits transcription.

reproductive isolating mechanism

Any biological factor or mechanism that prevents gene exchange.

repulsion (trans) configuration

Arrangement of two linked genes in which each of a homologous pair of chromosomes contains one wild-type (dominant) allele and one mutant (recessive) allele.

response element

DNA sequence shared by the promoters or enhancers of several eukaryotic genes to which a regulatory protein can bind to stimulate the coordinate transcription of those genes.

response to selection

The extent to which a characteristic subjected to selection changes in one generation; equals the selection differential times the narrow-sense heritability.

restriction endonuclease

See **restriction enzyme**.

restriction enzyme

Enzyme that recognizes particular base sequences in DNA and makes double-stranded cuts at those sequences; also called a **restriction endonuclease**.

restriction mapping

Method of determining the locations of sites cut by restriction enzymes in a piece of DNA.

restriction-modification system

A type of bacterial defense that uses restriction enzymes to cleave foreign DNA. The bacteria protects its own DNA from cleavage by modifying the sequences recognized by the restriction enzymes.

retrotransposon

Type of transposable element in eukaryotic cells that possesses some characteristics of retroviruses and transposes through an RNA intermediate.

retrovirus
Virus that injects its RNA genome into a host cell, where reverse transcription produces a complementary, double-stranded DNA molecule from the RNA template; the DNA copy then integrates into the host chromosome to form a provirus.
reverse duplication
Duplication of a chromosome segment in which the sequence of the duplicated segment is inverted relative to the sequence of the original segment.
reverse genetics
A molecular approach to the study of gene function that begins with a genotype (a DNA sequence) and proceeds to the phenotype by altering the sequence or by inhibiting its expression.
reverse mutation (reversion)
Mutation that changes a mutant phenotype back into the wild type.
reverse transcriptase
Enzyme that can synthesize complementary DNA from either an RNA or a DNA template.
reverse transcription
Synthesis of DNA from an RNA template.
reverse-transcription PCR
Technique that amplifies sequences corresponding to RNA; reverse transcriptase is used to convert RNA into complementary DNA, which can then be amplified by the usual polymerase chain reaction.
reversion
See **reverse mutation**.
rho-dependent terminator
Sequence in bacterial DNA that requires the presence of the rho factor to terminate transcription.
rho factor (ρ)
A protein that binds to bacterial RNA polymerase and facilitates the termination of transcription of some genes.
rho-independent terminator
Sequence in bacterial DNA that does not require the presence of the rho factor to terminate transcription.
ribonucleoside triphosphate (rNTP)
Substrate of RNA synthesis; consists of a ribose sugar and a base (a nucleoside) attached to three phosphate groups. In transcription, two of the phosphates are cleaved, producing an RNA nucleotide.
ribonucleotide
Basic building block of RNA, consisting of ribose, a phosphate group, and a nitrogenous base.
ribose
Five-carbon sugar in RNA; has a hydroxyl group attached to the 2′-carbon atom.
ribosomal RNA (rRNA)

RNA molecule that is a structural component of the ribosome.

riboswitch

Regulatory sequence in an RNA molecule. When an inducer molecule binds to the riboswitch, this changes the configuration of the RNA molecule and alters the expression of the RNA, usually by affecting the termination of transcription or by affecting translation.

ribozyme

RNA molecule that can act as a biological catalyst.

RNA-coding region

Sequence of DNA nucleotides that is copied into an RNA molecule.

RNA editing

Process in which the protein-coding sequence of an mRNA molecule is altered after transcription, so that the translated protein has an amino acid sequence that differs from that encoded by the gene.

RNA-induced silencing complex (RISC)

Complex of a small interfering RNA (siRNA) or microRNA (miRNA) with proteins that can cleave mRNA, leading to degradation of the mRNA or repression of its translation.

RNA interference (RNAi)

Process in which cleavage of double-stranded RNA produces small RNAs (siRNAs or miRNAs) that bind to mRNAs containing complementary sequences and bring about their cleavage and degradation.

RNA polymerase

Enzyme that synthesizes RNA from a DNA template during transcription.

RNA polymerase I

Eukaryotic RNA polymerase that transcribes large ribosomal RNA molecules.

RNA polymerase II

Eukaryotic RNA polymerase that transcribes pre-mRNA, snoRNAs, some miRNAs, and some snRNAs.

RNA polymerase III

Eukaryotic RNA polymerase that transcribes small ribosomal molecules—tRNAs, small rRNAs, some miRNAs, and some snRNAs.

RNA polymerase IV

RNA polymerase that transcribes RNAs that play a role in chromatin structure in plants.

RNA polymerase V

RNA polymerase that transcribes RNAs that play a role in heterochromatin formation in plants.

RNA replication

Process in some viruses by which RNA is synthesized from an RNA template.

RNA sequencing

A method in which cDNA molecules are copied from the RNA molecules present in a cell and sequenced.

RNA splicing
Process by which introns are removed from RNA and exons are joined together.
Robertsonian translocation
Translocation in which the long arms of two acrocentric chromosomes become joined to a common centromere, generating a metacentric chromosome with two long arms and another chromosome with two very short arms.
rolling-circle replication
Replication of circular DNA that is initiated by a break in one of the nucleotide strands, producing a double-stranded circular DNA molecule and a single-stranded linear DNA molecule, the latter of which may circularize and serve as a template for the synthesis of a complementary strand.
rooted tree
Phylogenetic tree in which one node represents a common ancestor to all other organisms (nodes) on the tree. In a rooted tree, all the organisms depicted have a common ancestor.
R plasmid
Plasmid possessing genes that confer antibiotic resistance on any cell that contains the plasmid.
sample
Subset used to describe a population.
sampling error
Deviations from expected ratios due to chance when the sample size is small.
secondary immune response
Generation of a clone of cells when a memory cell encounters an antigen; provides long-lasting immunity.
secondary oocyte
One of the products of meiosis I in oogenesis; receives most of the cytoplasm.
secondary spermatocyte
Product of meiosis I in male animals.
secondary structure of a protein
Regular folding arrangement of amino acids in a protein. Common secondary structures found in proteins include the alpha helix and the beta pleated sheet.
second polar body
One of the products of meiosis II in oogenesis; contains a set of chromosomes but little of the cytoplasm.
segmental duplications
Duplicated chromosome segments larger than 1000 bp.
segmentation genes
Set of about 25 genes in fruit flies that control the differentiation of the embryo into individual segments. These genes affect the number and organization of the segments, and mutations in them usually disrupt whole sets of segments.
segment-polarity genes

Set of segmentation genes in fruit flies that affect the orientation of segments. Mutations in segment-polarity genes cause part of each segment to be deleted and replaced by a mirror image of part or all of an adjacent segment.

segregation

See **principle of segregation**.

selection coefficient

Measure of the relative intensity of selection against a genotype; equals 1 minus *W* (fitness value).

selection differential

Difference between the mean phenotype of the selected parents and the mean phenotype of the original population.

semiconservative replication

Replication in which the two nucleotide strands of DNA separate, and each serves as a template for the synthesis of a new strand. All DNA replication is semiconservative.

sense codon

Codon that specifies an amino acid in a protein.

separase

Molecule that cleaves cohesin molecules, which hold the sister chromatids together.

sequencing coverage

The average number of times a nucleotide in the genome is sequenced in a sequencing study.

sequential hermaphroditism

Phenomenon in which the sex of an individual organism changes in the course of its lifetime; the organism is male at one age or developmental stage and female at a different age or stage.

70S initiation complex

Final complex formed in the initiation of translation in bacterial cells; consists of the small and large subunits of the ribosome, mRNA, and initiator tRNA charged with fMet.

sex

Sexual phenotype: male or female.

sex chromosomes

Chromosomes that differ in number or morphology in males and females.

sex determination

Specification of sex (male or female). Sex-determining mechanisms include chromosomal, genic, and environmental sex-determining systems.

sex-determining region Y (*SRY*) gene

The male-determining gene in mammals, located on the Y chromosome.

sex-influenced characteristic

Characteristic encoded by autosomal genes that are more readily expressed in one sex. For example, an autosomal dominant gene may have higher penetrance in males than in females, or an autosomal gene may be dominant in males but recessive in females.

sex-limited characteristic

Characteristic encoded by autosomal genes and expressed in only one sex. Both males and females carry genes for sex-limited characteristics, but the characteristics appear in only one of the sexes.

sex-linked characteristic

Characteristic determined by a gene or genes on sex chromosomes.

shelterin

Multiprotein complex that binds to telomeres and protects the ends of the DNA from being inadvertently repaired as a double-stranded break in the DNA.

Shine–Dalgarno sequence

Consensus sequence found in the bacterial 5′ untranslated region of mRNA; contains the ribosome-binding site.

short interspersed element (SINE)

Short DNA sequence repeated many times and interspersed throughout the genome.

short tandem repeat (STR)

See **microsatellite**.

sigma (σ) factor

Subunit of bacterial RNA polymerase that allows the RNA polymerase to recognize a promoter and initiate transcription.

signal sequence

Sequence of 15 to 30 amino acids found at the amino end of some eukaryotic proteins that directs the protein to a specific location in the cell; usually cleaved from the protein.

signal-transduction pathway

System in which an external signal (initiated by a hormone or growth factor) triggers a cascade of intracellular reactions that ultimately produce a specific response.

silencer

Sequence that has many of the properties possessed by an enhancer but represses transcription.

silent mutation

Change in the nucleotide sequence of DNA that does not alter the amino acid sequence of a protein.

SINE

See **short interspersed element**.

single-nucleotide polymorphism (SNP)

A site in the genome where individual members of a species differ in a single base pair.

single-strand-binding (SSB) protein

Protein that attaches tightly to the exposed single-stranded DNA during replication and prevents the formation of secondary structures that would interfere with replication.

sister chromatids

Two copies of a chromosome that are held together at the centromere. Each sister chromatid consists of a single molecule of DNA.

site-directed mutagenesis
Targeted mutagenesis technique used in bacteria in which a short sequence of nucleotides is cut out with restriction enzymes and replaced with a synthetic oligonucleotide.
small interfering RNA (siRNA)
Single-stranded RNA molecule (usually 21 to 25 nucleotides in length) produced by the cleavage and processing of double-stranded RNA that binds to complementary sequences in mRNA and brings about the cleavage and degradation of the mRNA. Some siRNAs bring about changes in chromatin structure.
small nuclear ribonucleoprotein (snRNP)
Structure found in the nuclei of eukaryotic cells that consists of small nuclear RNA (snRNA) and protein; functions in the processing of pre-mRNA.
small nuclear RNA (snRNA)
Small RNA molecule found in the nuclei of eukaryotic cells; functions in the processing of pre-mRNA.
small nucleolar RNA (snoRNA)
Small RNA molecule found in the nuclei of eukaryotic cells; functions in the processing of rRNA and in the assembly of ribosomes.
small ribosomal subunit
The smaller of the two subunits of a functional ribosome.
somatic-cell hybridization
Fusion of somatic cells of different types. Used for positioning genes on chromosomes.
somatic hypermutation
High rate of somatic mutation, such as that in genes encoding antibodies.
somatic mutation
Mutation that arises in somatic cells and does not give rise to gametes.
somatic recombination
Recombination in maturing lymphocytes among segments of genes that encode antibodies and T-cell receptors.
SOS system
System of proteins and enzymes that allows a cell to replicate its DNA in the presence of a distortion in DNA structure; makes numerous mistakes in replication and increases the rate of mutation.
Southern blotting
Technique by which DNA is transferred from a gel to a solid support, such as a nitrocellulose or nylon filter.
specialized transduction
Transduction in which genes near special sites on the bacterial chromosome are transferred from one bacterium to another; requires lysogenic bacteriophages.
speciation

Process by which new species arise. *See also* **allopatric speciation**, **sympatric speciation**.

species

Term applied to different kinds or types of living organisms. *See also* **biological species concept**.

spermatid

Immediate product of meiosis II in spermatogenesis; matures to sperm.

spermatogenesis

Production of gametes in a male animal; takes place in the testes.

spermatogonium

Diploid cell in the testis; capable of undergoing meiosis to produce a sperm.

S (synthesis) phase

Stage of interphase in the cell cycle. In S phase, DNA replicates.

spindle-assembly checkpoint

Checkpoint in the cell cycle near the end of metaphase; after this checkpoint has been passed, the cell enters anaphase.

spindle microtubule

Microtubule that moves chromosomes in mitosis and meiosis.

spindle pole

Point from which spindle microtubules radiate.

spliceosome

Large complex consisting of several RNAs and many proteins that splices protein-encoding pre-mRNA; contains five small nuclear ribonucleoprotein particles (U1, U2, U4, U5, and U6).

spontaneous mutation

Mutation that arises from natural changes in DNA structure or from errors in replication.

sporophyte

Diploid phase of the life cycle in plants.

SR proteins

A group of serine- and arginine-rich proteins that regulate alternative splicing of pre-mRNA.

standard deviation

Statistic that describes the variability of a group of measurements; the square root of the variance.

start codon

See **initiation codon**.

stem cell

Undifferentiated cell that is capable of forming every type of cell in an organism.

sticky end

See **cohesive end**.

stop (termination or nonsense) codon

Codon in mRNA that signals the end of translation. The three common stop codons are UAA, UAG, and UGA.

strand slippage

Slipping of the template and newly synthesized strands in replication in which one of the strands loops out from the other and nucleotides are inserted or deleted on the newly synthesized strand.

structural gene

DNA sequence that encodes a protein that functions in metabolism or biosynthesis or that has a structural role in the cell.

structural genomics

Study of the organization and sequence of the genetic information contained within a genome, providing the basic DNA sequence information that is used in functional and evolutionary studies; sometimes used by protein chemists to refer to the determination of the three-dimensional structure of proteins.

structural variants

Collective term for chromosome rearrangements and copy-number variations.

submetacentric chromosome

Chromosome in which the centromere is displaced toward one end, producing a short arm and a long arm.

supercoiling

Tertiary structure that forms when strain is placed on a DNA helix by overrotation or underrotation. *See also* **positive supercoiling**, **negative supercoiling**.

super-enhancer

A number of enhancers clustered together that stimulate high levels of transcription.

suppressor mutation

Mutation that hides or suppresses the effect of another mutation at a nucleotide site that is distinct from the site of the original mutation.

sympatric speciation

Speciation that arises in the absence of any geographic barrier to gene flow, in which reproductive isolating mechanisms evolve within a single population.

synapsis

Close pairing of homologous chromosomes.

synaptonemal complex

Three-part structure that develops between synapsed homologous chromosomes.

synonymous codons

Different codons that specify the same amino acid.

synthetic biology

A field that seeks to design organisms that might provide functions useful to humanity.

tag single-nucleotide polymorphism (tag-SNP)

Single-nucleotide polymorphism used to identify a haplotype.

tandem duplication

Chromosome rearrangement in which a duplicated chromosome segment is adjacent to the original segment.

tandem repeats
Type of moderately repetitive DNA in which sequences are repeated one after another; tend to be clustered at specific locations on a chromosome.
***Taq* polymerase**
DNA polymerase commonly used in PCR reactions. Isolated from the bacterium *Thermus aquaticus*, the enzyme is stable at high temperatures, so it is not denatured during the strand-separation step of the cycle.
targeted mutagenesis
Process by which mutations are induced at specific locations to study their effects.
TATA-binding protein (TBP)
Polypeptide chain found in several different transcription factors that recognizes and binds to the TATA box.
TATA box
Consensus sequence (TATAAA) commonly found in eukaryotic RNA polymerase II promoters; usually located –25 to –30 bp upstream of the transcription start site. The TATA box determines the start point for transcription.
T cell
Specialized lymphocyte that matures in the thymus and responds to antigens found on the surfaces of the body's own cells; responsible for cellular immunity.
T-cell receptor
Protein found on the surface of a T cell that simultaneously binds a foreign antigen and a self-antigen.
telocentric chromosome
Chromosome in which the centromere is at or very near one end.
telomerase
Ribonucleoprotein enzyme that replicates the ends (telomeres) of eukaryotic chromosomes. The RNA part of the enzyme has a template that is complementary to repeated sequences in the telomere and pairs with them, providing a template for the synthesis of additional copies of the repeats.
telomere
Stable end of a eukaryotic chromosome.
telomeric sequence
Sequence found at the ends of a chromosome; consists of many copies of short, simple sequences repeated one after the other.
telophase
Stage of mitosis in which the chromosomes arrive at the spindle poles, the nuclear membrane re-forms, and the chromosomes relax and lengthen.
telophase I
Stage of meiosis I in which chromosomes arrive at the spindle poles.

telophase II
Stage of meiosis II in which chromosomes arrive at the spindle poles.
temperate phage
Bacteriophage that can undergo the lysogenic cycle, in which the phage DNA integrates into the bacterial chromosome and remains in an inactive state.
temperature-sensitive allele
An allele that is functional at only certain temperatures.
template strand
The nucleotide strand that is used as a template during transcription. The RNA synthesized during transcription is complementary and antiparallel to the DNA template strand.
temporal isolation
Reproductive isolation in which the reproduction of different species takes place at different times of the year; exemplified by some species of plants that do not exchange genes because they flower at different times of the year.
terminal inverted repeats
Sequences found at both ends of a transposable element that are inverted complements of one another.
termination codon
See **stop codon**.
terminator
Sequence of DNA nucleotides that brings about the termination of transcription.
tertiary structure of a protein
Higher-order folding of amino acids in a protein to form the overall three-dimensional shape of the molecule.
testcross
Cross between an individual with an unknown genotype and an individual with the homozygous recessive genotype.
tetrad
See **bivalent**.
tetrasomy
Presence of two extra copies of a chromosome ($2n + 2$).
theory of clonal selection
Theory that explains the generation of primary and secondary immune responses; proposes that the binding of a lymphocyte to an antigen stimulates the cell to divide, giving rise to a clone of genetically identical cells, all of which are specific for the antigen.
theta replication
Replication of circular DNA that is initiated by the unwinding of the two nucleotide strands, producing a replication bubble. Unwinding continues at one or both ends of the bubble, making it

progressively larger. DNA replication on both of the template strands is simultaneous with unwinding until the two replication forks meet.

30S initiation complex

Initial complex formed in the initiation of translation in bacterial cells; consists of the small ribosomal subunit, the mRNA, the initiator tRNA with its amino acid (fMet-$tRNA_i^{fMe}$), one molecule of GTP, and several initiation factors.

three-point testcross

Cross between an individual heterozygous at three loci and an individual homozygous for recessive alleles at those loci.

3′ end

End of a polynucleotide chain at which an OH group is attached to the 3′-carbon atom of the sugar in the nucleotide.

3′, 5′-cyclic adenosine monophosphate (cAMP)

Modified nucleotide that functions in catabolite repression. Low levels of glucose stimulate high levels of cAMP; cAMP then attaches to CAP, which binds to the promoters of certain operons and stimulates transcription.

3′ splice site

The 3′ end of an intron where cleavage takes place in RNA splicing.

3′ untranslated region (3′ UTR)

Sequence of nucleotides at the 3′ end of mRNA that is not translated into amino acids; affects the stability of the mRNA and helps regulate the translation of the mRNA protein-coding sequence.

threshold characteristic

Characteristic that has only two phenotypes (presence and absence) but whose expression depends on an underlying susceptibility that varies continuously.

thymine (T)

Pyrimidine base in DNA, but not in RNA.

Ti plasmid

Large plasmid isolated from the bacterium *Agrobacterium tumefaciens* and used to transfer genes to plant cells.

topoisomerase

Enzyme that adds or removes rotations in a DNA helix by temporarily breaking nucleotide strands; controls the degree of DNA supercoiling.

topologically associated domains (TADs)

Large regions of spatially interacting chromatin; may contain tens of thousands to millions of bp of DNA. Within a TAD, regions of chromatin lie in close proximity and interact with one another but are separated in space from other TADs. Help to control gene expression in eukaryotic cells.

totipotency

The potential of a cell to develop into any other cell type.

trans configuration

See **repulsion configuration**.

transcription

Process by which RNA is synthesized from a DNA template.

transcription activator–like effector nuclease (TALEN)

An engineered nuclease in which a protein of a type that normally binds to sequences in promoters is attached to a restriction enzyme.

transcription bubble

Region of a DNA molecule that has unwound to expose a single-stranded template, which is being transcribed into RNA.

transcription factor

Protein that binds to DNA sequences in eukaryotic cells and affects levels of transcription.

transcription start site

The first DNA nucleotide that is transcribed into an RNA molecule.

transcription unit

Sequence of nucleotides in DNA that encodes a single RNA molecule and the sequences necessary for its transcription; normally contains a promoter, an RNA-coding sequence, and a terminator.

transcriptome

Set of all RNA molecules transcribed from a genome.

transcriptomics

Study of the expression of the genome.

transducing phage

Phage that contains a piece of the chromosome of its bacterial host inside the phage coat.

transductant

Bacterial cell that has received genes from another bacterium through transduction.

transduction

Type of gene exchange that takes place when a virus carries genes from one bacterium to another. After it is inside the cell, the newly introduced DNA may undergo recombination with the bacterial chromosome.

transesterification

Chemical reaction in some RNA-splicing reactions.

transfer–messenger RNA (tmRNA)

An RNA molecule that has properties of both mRNA and tRNA; functions in rescuing ribosomes that are stalled at the end of an mRNA molecule.

transfer RNA (tRNA)

RNA molecule that carries an amino acid to the ribosome and transfers it to a growing polypeptide chain in translation.

transfer RNA introns

A class of introns in tRNA genes whose splicing relies on enzymes.

transformant

Cell that has received genetic material through transformation.

transformation

Mechanism by which DNA found in the environment is taken up by a cell. After transformation, recombination may take place between the introduced genes and those of the bacterial chromosome.

transforming principle

Substance responsible for transformation. DNA is the transforming principle.

transgene

Foreign gene or other DNA fragment carried in germ-line DNA.

transgenic mouse

Mouse whose genome contains a foreign gene or genes added by employing recombinant DNA methods.

transition

Base substitution in which a purine is replaced by a different purine or a pyrimidine is replaced by a different pyrimidine.

translation

Process by which a protein is assembled from information contained in mRNA.

translesion DNA polymerase

Specialized DNA polymerase that is able to replicate DNA through distorted structures and bulky lesions that halt other DNA polymerases; often makes more errors during DNA synthesis than other DNA polymerases.

translocation

(1) Movement of genetic material between nonhomologous chromosomes or within the same chromosome. (2) Movement of a ribosome along mRNA in the course of translation.

translocation carrier

Individual organism heterozygous for a chromosome translocation.

transmission genetics

Field of genetics that encompasses the basic principles of heredity and how traits are passed from one generation to the next.

transposable element

DNA sequence capable of moving from one site to another within the genome through a mechanism that differs from that of homologous recombination.

transposase

Enzyme encoded by many types of transposable elements that is required for their transposition. The enzyme makes single-strand breaks at each end of the transposable element and on either side of the target sequence where the element inserts.

transposition

Movement of a transposable element from one location to another. *See also* **replicative transposition**, **nonreplicative transposition**.

trans-splicing

Process of splicing together exons from two or more pre-mRNAs.

transversion

Base substitution in which a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine.

triplet code

Refers to the fact that three nucleotides encode each amino acid in a protein.

triple-X syndrome

Human condition in which cells contain three X chromosomes. A person with triple-X syndrome has a female phenotype with no distinctive features other than a tendency to be tall and thin; a few such females are sterile, but many menstruate regularly and are fertile.

trisomy

Presence of an extra copy of a chromosome ($2n + 1$).

trisomy 8

Presence of three copies of chromosome 8; in humans, results in intellectual disability, contracted fingers and toes, low-set malformed ears, and a prominent forehead.

trisomy 13

See **Patau syndrome**.

trisomy 18

See **Edwards syndrome**.

trisomy 21

See **Down syndrome**.

tRNA charging

Chemical reaction in which an aminoacyl-tRNA synthetase attaches an amino acid to its corresponding tRNA.

tRNA-modifying enzyme

Enzyme that creates a modified base in tRNA by catalyzing a chemical change in the standard base.

tubulin

Protein found in microtubules.

tumor-suppressor gene

Gene that normally inhibits cell division. Recessive mutations in such genes often contribute to cancer.

Turner syndrome

Human condition in which cells contain a single X chromosome and no Y chromosome (XO). People with Turner syndrome are female in appearance and have underdeveloped female secondary sex characteristics; most are sterile but have normal intelligence.

two-dimensional polyacrylamide gel electrophoresis (2D-PAGE)
Method for separating proteins in which the proteins are separated in one dimension by charge, separated in a second dimension by mass, and then stained. Each of the resulting spots is proportional to the amount of protein present.

two-point testcross
Cross between an individual heterozygous at two loci and an individual homozygous for recessive alleles at those loci.

ultrasonography
Procedure for visualizing a fetus in which high-frequency sound is beamed into the uterus; sound waves that encounter dense tissue bounce back and are transformed into a picture of the fetus.

unbalanced gamete
Gamete that has a variable number of chromosomes; some chromosomes may be missing and others may be present in more than one copy.

underdominance
Selection in which the heterozygote has lower fitness than either homozygote.

unequal crossing over
Misalignment of the two DNA molecules during crossing over, resulting in one DNA molecule with an insertion and the other with a deletion.

uniparental disomy
Inheritance of both chromosomes of a homologous pair from a single parent.

unique-sequence DNA
DNA sequence that is present only once or, at most, a few times in the genome.

universal genetic code
Refers to the fact that particular codons specify the same amino acids in almost all organisms.

upstream element
Consensus sequence found in some bacterial promoters that contains a number of A–T pairs and is located about 40 to 60 bp upstream of the transcription start site.

uracil (U)
Pyrimidine base in RNA, but not normally in DNA.

variance
Statistic that describes the variability of a group of measurements.

virulent phage
Bacteriophage that reproduces strictly through the lytic cycle and kills its host cell.

virus
Noncellular replicating structure consisting of nucleic acid surrounded by a protein coat; can replicate only within a host cell.

Western blotting
Process by which protein is transferred from a gel to a solid support, such as a nitrocellulose or nylon membrane.

whole-genome shotgun sequencing
Method of sequencing a genome in which sequenced fragments are assembled into the correct sequence in contigs by using the overlaps in sequence.
wild type
The trait or allele that is most commonly found in natural (wild) populations.
wobble
Base pairing between codon and anticodon in which there is nonstandard pairing, usually at the third ($3'$) position of the codon; allows more than one codon to pair with the same anticodon.
X-inactivation center
Segment of the X chromosome at which inactivation of one X chromosome is initiated in female cells.
X-linked characteristic
Characteristic determined by a gene or genes on the X chromosome.
X-ray diffraction
Method for analyzing the three-dimensional shape and structure of a chemical substance in which crystals of the substance are bombarded with X-rays, which hit the crystals, bounce off, and produce a pattern of spots on a detector. The pattern of the spots produced on the detector provides information about the molecular structure.
yeast artificial chromosome (YAC)
Cloning vector consisting of a DNA molecule with a yeast origin of replication, a pair of telomeres, and a centromere. YACs can carry very large DNA fragments (as large as 600 kb) and can replicate and segregate the same way as yeast chromosomes do.
Y-linked characteristic
Characteristic determined by a gene or genes on the Y chromosome.
Z-DNA
Secondary structure of DNA characterized by 12 bases per turn, a left-handed helix, and a sugar–phosphate backbone that zigzags back and forth.
zinc-finger nuclease (ZFN)
An engineered nuclease consisting of an array of zinc-finger domains attached to a restriction enzyme.
zygotene
Second substage of prophase I in meiosis. In zygotene, chromosomes enter into synapsis.

Answers to Selected Problems

Chapter 1

1. In the Hopi culture, people with albinism were considered special and given special status. Because extensive exposure to sunlight could be damaging or deadly, Hopi males with albinism did no agricultural work. Males with albinism had more children, thus increasing the frequency of the albino mutation. Finally, the small size of the Hopi population may have helped increase the frequency of the albino mutation, owing to chance.

17. Evolution is genetic change over time. For evolution to occur, genetic variation must first arise, and then evolutionary forces change the proportions of genetic variants over time. Genetic variation is, therefore, the basis of all evolutionary change.

18.

a. Transmission genetics;

b. population genetics;

c. population genetics;

d. molecular genetics;

e. molecular genetics;

f. transmission genetics.

22. Genetics is "old" in the sense that humans have been aware of hereditary principles for thousands of years, and have applied them since the beginning of agriculture and the domestication of plants and animals. It is very "young" in the sense that the fundamental principles were not uncovered until Mendel's time, and the discovery of the structure of DNA and recombinant DNA techniques occurred only within the last 60 years.

23.

a. Germ-plasm theory;

b. preformationism;

c. inheritance of acquired characteristics;

d. pangenesis.

25.

a. Both cell types have genomes encoded in nucleic acids, and the two cell types have similar machinery for the replication, transcription, and translation of those genomes. Eukaryotic cells have a nucleus containing chromosomal DNA and possess internal membrane-bounded organelles; prokaryotic cells have neither of these features.

b. A gene is a basic unit of hereditary information. Alleles are alternative forms of a gene.

c. The genotype is the set of genes or alleles inherited by an organism from its parent(s). The expression of the genes of a particular genotype, through interaction with environmental factors, produces the phenotype, an observable trait.

d. Both are nucleic acid polymers that encode genetic information. RNA contains a ribose sugar, whereas DNA contains a deoxyribose sugar. RNA contains uracil as one of the four bases, whereas DNA contains thymine. The other three bases are common to both DNA and RNA. Finally, DNA usually consists of two complementary nucleotide strands, whereas RNA is single stranded.

e. Chromosomes are structures consisting of DNA and associated proteins. The DNA contains the genetic information.

26.

Type of albinism	Phenotype	Gene mutated
OCA2	Pigment reduced in skin, hair, and eyes, but small amount of pigment acquired with age; visual problems	*OCA2*
OCA1B	General absence of pigment in hair, skin, and eyes, but may be small amount of pigment; does not vary with age; visual problems	Tyrosinase gene (*TYR*)
OCA1A	Complete absence of pigment; visual problems	Tyrosinase gene (*TYR*)
OCA3	Some pigment present, but sun sensitivity and visual problems	Tyrosinase-related protein 1 gene (*TYRP1*)
OASD	Lack of pigment in the eyes and deafness later in life	Unknown

OA1	Lack of pigment in the eyes but normal elsewhere	*GPR143*
ROCA	Bright copper red coloration in skin and hair of Africans; dilution of color in iris	Tyrosinase-related protein 1 gene (*TYRP1*)
OCA4	Reduced pigmentation	*MATP*

29. All genomes must have the ability to store complex information and to vary. The genetic material of any organism must be stable, replicated precisely, and transmitted faithfully to the progeny but must be capable of mutating.

Chapter 2

20.

a. The two chromatids of a chromosome

b. The two chromosomes of a homologous pair

c. Cohesin

d. The enzyme separase

e. The hands of the two blind men

f. If one man failed to grasp his sock, use of the knife to cut the string holding them together would be difficult. The two socks of a pair would not be separated, and both would end up in one man's bag. Similarly, if each chromatid is not attached to a spindle microtubule, and if the two chromatids are not pulled in opposite directions, they will not separate, and both will migrate to the same cell. This cell will have two copies of one chromosome.

24.

Stage	Number of cells at each stage	Proportion of cells at each stage	Average duration (hours)
Interphase	160	0.80	19.2
Prophase	20	0.10	2.4
Prometaphase	6	0.03	0.72
Metaphase	2	0.01	0.24
Anaphase	7	0.035	0.84
Telophase	5	0.025	0.6
Totals	200	1.000	24.00

The average duration of M phase can be determined by adding up the hours spent in each stage of mitosis. In these cells, M phase lasts 4.8 hours. Metaphase requires 0.24 hours, or 14.4 minutes.

29.

a. 12 chromosomes and 24 DNA molecules;

b. 12 chromosomes and 24 DNA molecules;

c. 12 chromosomes and 24 DNA molecules;

d. 12 chromosomes and 24 DNA molecules;

e. 12 chromosomes and 12 DNA molecules;

f. 6 chromosomes and 12 DNA molecules;

g. 12 chromosomes and 12 DNA molecules;

h. 6 chromosomes and 6 DNA molecules.

31.

a. The diploid number of chromosomes is 6.

b. The left-hand cell is in anaphase I of meiosis the middle cell is in anaphase of mitosis and the right-hand cell is in anaphase II of meiosis.

c. Anaphase I, 6 chromosomes and 12 DNA molecules; anaphase of mitosis, 12 chromosomes and 12 DNA molecules; anaphase II of meiosis, 6 chromosomes and 6 DNA molecules.

32.

a. 7.3 pg;

b. 14.6 pg;

c. 14.6 pg;

d. 3.7 pg;

e. 14.6 pg;

f. 7.3 pg.

34.

a. If cohesin fails to form early in mitosis, the sister chromatids could separate prior to anaphase, resulting in improper segregation of chromosomes to daughter cells.

b. If shugoshin is absent during meiosis, the cohesin at the centromere could be broken, allowing for the separation of sister chromatids during anaphase I.

c. If shugoshin is not broken down, the cohesin at the centromere will remain protected from degradation. The intact cohesin will prevent the sister chromatids from separating during anaphase II of meiosis.

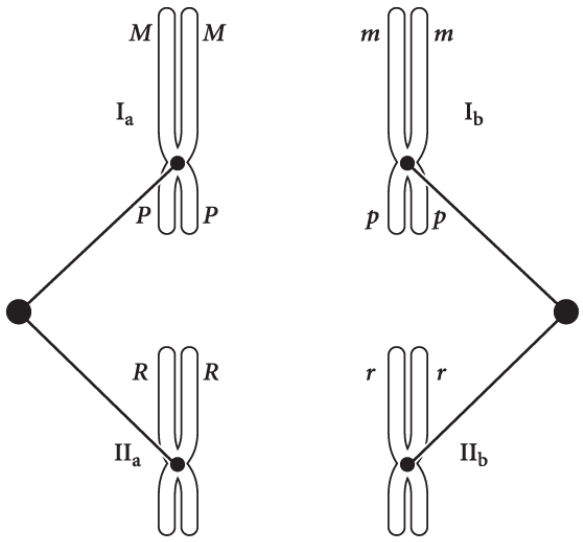
d. If separase is defective, homologous chromosomes and sister chromatids will not separate in meiosis and mitosis, resulting in some cells that have too few chromosomes and some cells that have too many chromosomes.

35. Prophase of mitosis: 24 chromosomes; prophase I of meiosis: 24 chromosomes.

37. More genetic variation in the house fly. The number of different combinations of chromosomes that are possible in the gametes is 2^n, where n is equal to the number of homologous pairs of chromosomes. For the fruit fly, $2^4 = 16$. For the house fly, $2^6 = 64$.

38.

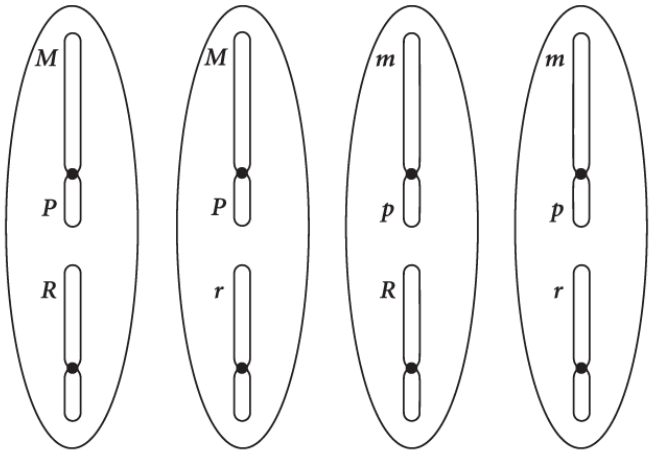
a. Metaphase



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

Description
The illustration shows two sets of long X chromosomes attached to spindle fibers; 1 subscript a and 2 subscript a attached to the fibers on left and 1 subscript b and 2 subscript b attached to the fibers on right. All the chromosomes have long upper arms and shorter lower arms. The genes are as follows: On 1 subscript a, the genes are big M big M big P big P and on 2 subscript a, the genes are big R big R. On 1 subscript b, the genes are small M small M small P small P and on 2 subscript a, the genes are small R small R.

b. Gametes



Pierce, *Genetics: A Conceptual Approach*, 7e © 2020 W. H. Freeman and Company

Description
Each gamete has two long rod-shaped chromosomes. All the chromosomes have long upper arms and shorter lower arms. The genes in each gamete are as follows:

First gamete: Chromosome 1, big M big P; chromosome 2, big R.

Second gamete: Chromosome 1, big M big P; chromosome 2, small R.

Third gamete: Chromosome 1, small M small P; Chromosome 2, big R.

Fourth gamete: Chromosome 1, small M small P; Chromosome 2, small R.

40.

Cell type	Number of chromosomes	Number of DNA molecules
(a) Spermatogonium	64	64
(b) First polar body	32	64
(c) Primary oocyte	64	128
(d) Secondary spermatocyte	32	64

42.

a. No. The first polar body and the secondary oocyte are the result of meiosis I, which produces two nonidentical cells. The first polar body and the secondary oocyte will contain only one member of each original chromosome pair. Additionally, crossing over that took place in prophase I will have generated new and different arrangements of genetic material on those chromatids that participated in crossing over.

b. No. The second polar body and the ovum will contain copies of the same members of the homologous pairs of chromosomes that separated in meiosis. However, because of crossing over, the sister chromatids that separated in anaphase II and gave rise to the ovum and second polar body are no longer identical.

46. Because meiosis takes place only in diploid cells, haploid male bees do not undergo meiosis. Male bees produce sperm only through mitosis. Haploid cells that divide mitotically produce haploid cells.

Chapter 3

13. Useful characteristics

- Easy to grow and maintain
- Grow rapidly, producing many generations in a short period
- Produce large numbers of offspring
- Have distinctive phenotypes that are easy to recognize

Examples of organisms that meet these criteria

- *Neurospora,* a fungus
- *Saccharomyces cerevisiae*, a yeast
- *Arabidopsis,* a plant
- *Caenorhabditis elegans,* a nematode
- *Drosophila melanogaster*, a fruit fly

14.

a. The parents are *RR* (orange fruit) and *rr* (cream fruit). All the F_1 are *Rr* (orange). The F_2 are 1 *RR* : 2 *Rr* : 1 *rr* and have a phenotypic ratio of 3 orange to 1 cream.

b. Half of the progeny are homozygous and have orange fruit (*RR*), and half are heterozygous and have orange fruit (*Rr*).

c. Half of the progeny are heterozygous and have orange fruit (*Rr*), and half are homozygous and have cream fruit (*rr*).

17.

a. Although the white female gave birth to the offspring, her eggs were produced by the ovary from the black female guinea pig. The transplanted ovary produced only eggs containing the allele for black coat color. Like most mammals, guinea pig females produce primary oocytes early in development, and thus the transplanted ovary already contained primary oocytes produced by the black female guinea pig.

b. *Ww*.

c. The production of black offspring suggests that the allele for black coat color was passed to the offspring from the transplanted ovary, in agreement with the germ-plasm theory. If pangenesis were correct, the offspring should have been white. The white-coat alleles would have traveled to the transplanted ovary and then into the white female's gametes. The absence of any white offspring indicates that pangenesis did not occur.

18.

a. Female parent is i^Bi^B; male parent is I^Ai^B.

b. Both parents are i^Bi^B.

c. Male parent is i^Bi^B; female parent is most likely I^AI^A. or possibly I^Ai^B, but a heterozygous female in this mating is unlikely to have produced eight blood type A kittens owing to chance alone.

d. Both parents are I^Ai^B.

e. Either both parents are I^AI^A or one parent is I^AI^A and the other parent is I^Ai^B. The blood type of the offspring does not allow a determination of the precise genotype of either parent.

f. Female parent is i^Bi^B; male parent is I^Ai^B.

21. Yes. The ram and the lamb must be homozygous for the normal allele (l) because both have the normal fleece phenotype. Because the lamb receives only a single allele (l) from the ram, the ewe must have contributed the other recessive l allele. Therefore, the ewe must be heterozygous for lustrous fleece. In summary:

$$\underset{(Ll)}{\text{Lustrous fleece ewe}} \times \underset{(ll)}{\text{Normal fleece ram}}$$

$$\downarrow$$

$$\underset{(ll)}{\text{Normal fleece lamb}}$$

22.

a. Sally is *Aa*, Sally's mother is *Aa*, Sally's father is *aa*, and Sally's brother is *aa*;

b. $^{1}/_{2}$;

c. $^{1}/_{2}$.

25.

a. $^{1}/_{6}$;

b. $^{1}/_{3}$;

c. $^{1}/_{2}$;

d. $^{5}/_{6}$.

26.

a. $^{1}/_{18}$;

b. $^{1}/_{36}$;

c. $^{11}/_{36}$;

d. $^{1}/_{6}$;

e. $^{1}/_{4}$;

f. $^{3}/_{4}$.

27.

a. $^{1}/_{128}$;

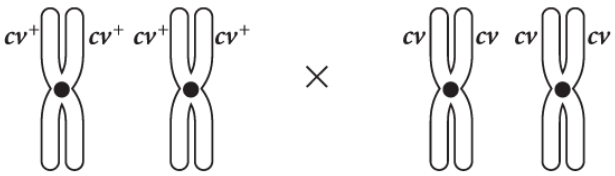
b. $^{1}/_{64}$;

c. $^{7}/_{128}$;

d. $^{35}/_{128}$;

e. $^{35}/_{128}$.

29. Parents:



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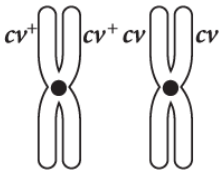
Description
Both the chromosomes in the first pair have the genotype small C small V superscript positive on each chromatid. Both the chromosomes in the second pair have the genotype small C small V on each chromatid.

F_1 generation:



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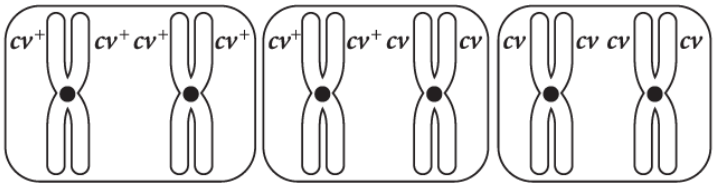
Description
The chromosome on the left has the genotype small C small V superscript positive on each chromatid. The chromosome on the right has small C small V on each chromatid.

F_2 generation:



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Description
Both the chromosomes in the first cell have the genotype small C small V superscript positive on each chromatid. In the second cell, the genotype of the first chromosome is small C small V superscript positive on each chromatid and the genotype of the second chromosome is small C small V on each chromatid. Both the chromosomes in the third cell have the genotype small C small V on each chromatid.

30.

a. G_1, one copy; G_2, two copies; metaphase of mitosis, two copies; metaphase I of meiosis, two copies; metaphase II of meiosis, two copies but in only half of the cells; after the second cytokinesis of meiosis, one copy, but only in half of the cells produced by meiosis (the remaining half will not contain the *B* allele).

b. G_1, two copies; G_2, four copies; metaphase of mitosis, four copies; metaphase I of meiosis, four copies; metaphase II of meiosis, two copies; after cytokinesis of meiosis, one copy.

33.

a. $^9/_{16}$ black and curled, $^3/_{16}$ black and normal, $^3/_{16}$ gray and curled, and $^1/_{16}$ gray and normal.

b. $^1/_4$ black and curled, $^1/_4$ black and normal, $^1/_4$ gray and curled, and $^1/_4$ gray and normal.

34.

a. $^1/_2\ (Aa) \times {}^1/_2\ (Bb) \times {}^1/_2\ (Cc) \times {}^1/_2\ (Dd) \times {}^1/_2\ (Ee) = {}^1/_{32}$.

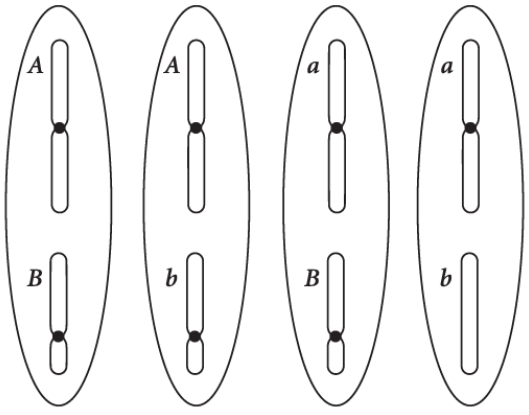
b. $^1/_2(Aa) \times {}^1/_2(bb) \times {}^1/_2(Cc) \times {}^1/_2(dd) \times {}^1/_4(ee) = {}^1/_{64}$.

c. $^1/_4(aa) \times {}^1/_2(bb) \times {}^1/_4(cc) \times {}^1/_2(dd) \times {}^1/_4(ee) = {}^1/_{256}$.

d. No offspring has this genotype.

37.

a. Gametes from *Aa Bb* individual:



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Description
Each gamete has two long linear rod-shaped chromosomes. All the first chromosomes in each gamete have equal upper and lower arms. The second chromosomes in first three gametes have short lower arms and longer upper arms, while the second chromosome in the fourth gamete has only one arm. The genes in each gamete are as follows:

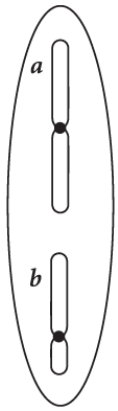
First gamete: Chromosome 1, big A; chromosome 2, big B.

Second gamete: Chromosome 1, big A; chromosome 2, small B.

Third gamete: Chromosome 1, small A; Chromosome 2, big B.

Fourth gamete: Chromosome 1, small A; Chromosome 2, small B.

Gametes from *aa bb* individual:



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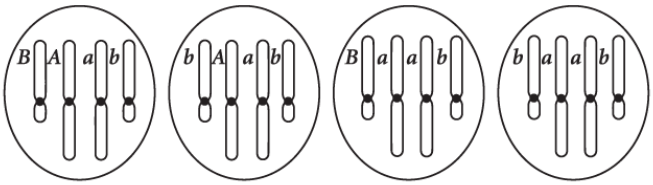
Description
The diagram shows one gamete with two long linear rod-shaped chromosomes. The first chromosome has equal arms, while the second chromosome has a short lower arm and longer upper arm. The genes in the gamete are as follows: Chromosome 1, small A; chromosome 2, small B.

b. Progeny at G_1:



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Description

Chromosomes 2 and 3 in each cell have equal arms, while the two other chromosomes (1 and 4) have one very short lower arm and one longer upper arm. The genes on chromosomes in each cell are as follows:

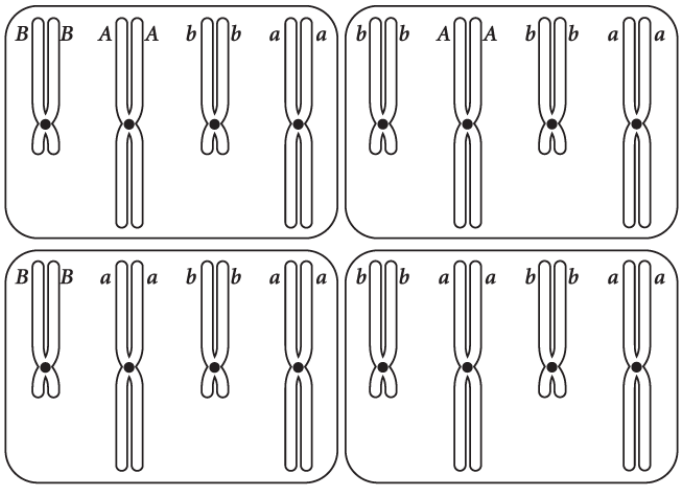
First gamete: Chromosome 1, big B; chromosome 2, big A; chromosome 3, small A; chromosome 4, small B.

Second gamete: Chromosome 1, small B; chromosome 2, big A; chromosome 3, small A; chromosome 4, small B.

Third gamete: Chromosome 1, big B; chromosome 2, small A; chromosome 3, small A; chromosome 4, small B.

Fourth gamete: Chromosome 1, small B; chromosome 2, small A; chromosome 3, small A; chromosome 4, small B.

Progeny at G_2:



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Description
Chromosomes 2 and 4 in each cell have equal arms, while the two other chromosomes (1 and 3) have one very short lower arm and one longer upper arm. The genes on chromosomes in each cell are as follows:

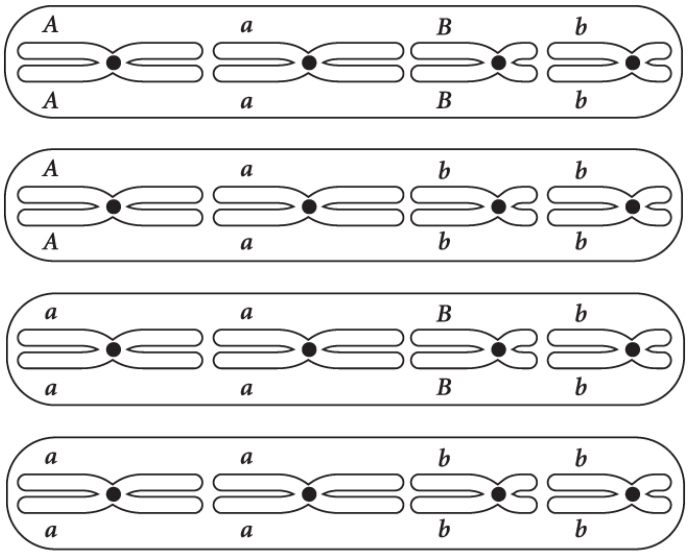
First gamete: Chromosome 1, big B big B; chromosome 2, big A big A; chromosome 3, small B small B; chromosome 4, small A small A.

Second gamete: Chromosome 1, small B small B; chromosome 2, big A big A; chromosome 3, small B small B; chromosome 4, small A small A.

Third gamete: Chromosome 1, big B big B; chromosome 2, small A small A; chromosome 3, small B small B; chromosome 4, small A small A.

Fourth gamete: Chromosome 1, small B small B; chromosome 2, small A small A; chromosome 3, small B small B; chromosome 4, small A small A.

Progeny at metaphase of mitosis:



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Description
Chromosomes 1 and 2 in each cell have equal arms, while the two other chromosomes (3 and 4) have one very short lower arm and one longer upper arm. The genes on chromosomes in each cell are as follows:

First gamete: Chromosome 1, big A big A; chromosome 2, small A small A; chromosome 3, big B big B; chromosome 4, small B small B.

Second gamete: Chromosome 1, big A big A; chromosome 2, small A small A; chromosome 3, small B small B; chromosome 4, small B small B.

Third gamete: Chromosome 1, small A small A; chromosome 2, small A small A; chromosome 3, big B big B; chromosome 4, small B small B.

Fourth gamete: Chromosome 1, small A small A; chromosome 2, small A small A; chromosome 3, small B small B; chromosome 4, small B small B.

The order of chromosomes on the metaphase plate can vary.

38.

a. The results are consistent with the burnsi phenotype being dominant to the pipiens phenotype.

b. Let B represent the burnsi allele and B^+ represent the pipiens allele.

$$\text{burnsi}\,(BB^+) \times \text{burnsi}\,(BB^+)$$
$$\text{burnsi}\,(BB^+) \times \text{pipiens}\,(B^+B^+)$$
$$\text{burnsi}\,(BB^+) \times \text{pipiens}\,(B^+B^+)$$

c. burnsi × burnsi $(BB^+ \times BB^+)$ cross : $\chi^2 = 3.26,\ \text{df} = 1, 0.1 > P > 0.05.$

burnsi × pipiens $(BB^+ \times B^+B^+)$ cross : $\chi^2 = 1.78, \text{df} = 1, P > 0.05.$

Second burnsi × pipiens $(BB^+ \times B^+B^+)$ cross : $\chi^2 = 0.46, \text{df} = 1, P > 0.05.$

41.

a. A chi-square test comparing the fit of the observed data with the expected 1 : 1 : 1 : 1 ratio yields χ^2 = 35, df = 3, P < 0.005.

b. Given the chi-square value, it is unlikely that chance produced the differences between the observed and expected ratios, indicating that the progeny are not in a 1 : 1 : 1 : 1 ratio.

c. The number of plants with the *cc ff* genotype is fewer than expected. Some of the plants with the *cc ff* genotype may have died before the progeny were counted.

43. The alleles for obesity from both laboratories are recessive. However, they are located at different gene loci that are independent of one another.

A cross between two obese mice from the first laboratory is

$$\begin{gathered}\text{Obese } (o_1o_1) \times \text{Obese } (o_1o_1) \\ \downarrow \\ \text{All obese } (o_1o_1)\end{gathered}$$

A cross between two obese mice from the second laboratory is

$$\begin{gathered}\text{Obese } (o_2o_2) \times \text{Obese } (o_2o_2) \\ \downarrow \\ \text{All obese } (o_2o_2)\end{gathered}$$

The cross between obese mice from the two laboratories is

$$\text{Obese mouse 1 } (o_1o_1O_2O_2) \times \text{Obese mouse 2 } (O_1O_1\ o_2o_2)$$
$$\downarrow$$
$$\text{All normal } (O_1o_1\ O_2o_2)$$

Chapter 4

4. XX-XY system: males are heterogametic and produce gametes with either an X chromosome or a Y chromosome. ZZ-ZW system: females are heterogametic and produce gametes with either a Z or a W chromosome.

15.

a. Female;

b. male;

c. male, sterile;

d. female;

e. male;

f. female;

g. metafemale;

h. male;

i. intersex;

j. female;

k. metamale, sterile;

l. metamale;

m. intersex.

17.

a. Female;

b. male;

c. male;

d. female;

e. male.

21. Her father's mother and her father's father, but not her mother's mother or her mother's father.

22. Bridges' exceptional white-eyed females were X^wX^wY, and the red-eyed males were X^+Y. The results of crossing such a white-eyed female with a red-eyed male are shown in **Figure 4.13**. Meiosis in this female generates 45% X^wY, 45% X^w, 5% X^wX^w, and 5% Y gametes. Meiosis in the red-eyed male generates 50% X^+ and 50% Y gametes.

	0.5 X^+	**0.5 Y**
0.45 X^wY	0.225 X^wX^+Y red-eyed females	0.225 X^wYY white-eyed males
0.45 X^w	0.225 X^wX^+ red-eyed females	0.225 X^wY white-eyed males
0.05 X^wX^w	0.025 $X^wX^wX^+$ metafemale, dies	0.025 X^wX^wY white-eyed females
0.05 Y	0.025 X^+Y red-eyed males	0.025 YY dies

23.

a. Yes;

b. yes;

c. no;

d. no.

24.

a. F_1 : $\frac{1}{2}X^+Y$ (gray males), $\frac{1}{2}X^+X^y$ (gray females); F_2 : $\frac{1}{4}X^+Y$ (gray males), $\frac{1}{4}X^yY$ (yellow males), $\frac{1}{4}X^+X^y$ (gray females), $\frac{1}{4}X^+X^+$ (gray females).

b. F_1 : $\frac{1}{2}X^yY$ (yellow males), $\frac{1}{2}X^+X^y$ (gray females); F_2 : $\frac{1}{4}X^+Y$ (gray males), $\frac{1}{4}X^yY$ (yellow males), $\frac{1}{4}X^+X^y$ (gray females), $\frac{1}{4}X^yX^y$ (yellow females).

c. F_2 : $\frac{1}{4}X^+Y$ (gray males), $\frac{1}{4}X^yY$ (yellow males), $\frac{1}{4}X^+X^+$ (gray females), $\frac{1}{4}X^+X^y$ (gray females).

d. F_3 : $\frac{1}{8}$ gray males, $\frac{3}{8}$ yellow males, $\frac{5}{16}$ gray females, and $\frac{3}{16}$ yellow females.

26. John has grounds for suspicion. The color-blind daughter must be X^cX^c and must have inherited a color-blindness allele from each parent. Because John has normal color vision, he must be X^+Y and, therefore, could not pass on a color-blindness allele to his daughter.

A remote alternative possibility is that the daughter is XO, having inherited a recessive color-blindness allele from her mother and no sex chromosome from her father. In that case, the daughter would have Turner syndrome and color blindness. Another unlikely possibility is that a new mutation in one of John's gametes produced a new, X-linked color-blindness allele that he passed on to the daughter.

If Cathy had given birth to a color-blind son, then John would have no grounds for suspicion. The son would have inherited John's Y chromosome and the color-blindness X chromosome from Cathy.

30. Because Bob must have inherited his Y chromosome from his father and his father has normal color vision, a nondisjunction event in the paternal lineage cannot account for Bob's genotype. Bob's mother must be heterozygous X^+X^c, because she has normal color vision, and she must have inherited an X^c chromosome from her color-blind father. For Bob to inherit two X^c chromosomes from his mother, the egg must have arisen from a nondisjunction in meiosis II. In meiosis I, the homologous X chromosomes separate, so one cell has the X^+ chromosome and the other has X^c. The failure of sister chromatids to separate in meiosis II would then result in an egg with two copies of X^c.

35. F_1 : $\frac{1}{2}\,Z^bZ^+$ (normal males), $\frac{1}{2}\,Z^bW$ (bald females).

F_2 : $\frac{1}{4}\,Z^+Z^b$ (normal males), $\frac{1}{4}\,Z^+W$ (normal females), $\frac{1}{4}\,Z^bZ^b$ (bald males), $\frac{1}{4}\,Z^bW$ (bald females)

40.

a. F_1: all males have miniature wings and red eyes ($X^mY\ s^+s$), and all females have long wings and red eyes ($X^+X^m\ s^+s$). F_2 : $\frac{3}{16}$ male, normal, red; $\frac{1}{16}$ male, normal, sepia; $\frac{3}{16}$ male, miniature, red; $\frac{1}{16}$ male, miniature, sepia; $\frac{3}{16}$ female, normal, red; $\frac{1}{16}$ female, normal, sepia; $\frac{3}{16}$ female, miniature, red; $\frac{1}{16}$ female, miniature, sepia.

b. F_1: all females have long wings and red eyes ($X^{m+}X^m\ s^+s$), and all males have long wings and red eyes ($X^{m+}Y\ s^+s$). F_2 : $\frac{3}{16}$ males, long wings, red eyes; $\frac{1}{16}$ males, long wings, sepia eyes; $\frac{3}{16}$ males, miniature wings, red eyes; $\frac{1}{16}$ males, miniature wings, sepia eyes; $\frac{6}{16}$ females, long wings, red eyes; $\frac{2}{16}$ females, long wings, sepia eyes.

42. Assuming that this form of color blindness is an X-linked trait, the mother must be X^cX^c, and the father must be X^+Y. Normally, all the sons should be color blind, and all the daughters should have normal color vision. The most likely way to have a daughter who is color blind would be for her not to have inherited an X^+ from her father. The observation that the color-blind daughter is short in stature and has failed to undergo puberty is consistent with Turner syndrome (XO). The color-blind daughter would then be X^cO.

44.

a. 1;

b. 0;

c. 0;

d. 1;

e. 1;

f. 2;

g. 0;

h. 2;

i. 3.

47.

a. X inactivation occurs randomly in each of the cells of the early embryo, and then is maintained in the mitotic progeny cells. The irregular patches of skin lacking sweat glands arose from skin precursor cells that inactivated the X chromosome with the normal allele.

b. The X-inactivation event occurs randomly in each of the cells of the early embryo. Even in identical twins, different skin precursor cells inactivate different X chromosomes, resulting in different distributions of patches lacking sweat glands.

48. If enlarged testes is an autosomal dominant trait, it appears only in males because only males have testes. An autosomal dominant trait would be passed from a heterozygous male to approximately half of his sons and could be passed through females. A Y-linked trait is passed from a father to all of his sons and cannot be passed through females. To determine whether the trait is Y linked or autosomal dominant, cross the male with enlarged testes with a female that

has no history of enlarged testes in her family (so that it is unlikely she is carrying a gene for enlarged testes). Then cross the F_1 females with normal males. If enlarged testes appear in some of the offspring, the trait cannot be Y linked.

Chapter 5

13.

a. The results of the crosses indicate that cremello and chestnut are pure-breeding traits (homozygous). Palomino is a heterozygous trait that produces a 1 : 2 : 1 ratio when palominos are crossed with each other. The simplest hypothesis consistent with these results is incomplete dominance, with palomino as the phenotype of the heterozygotes resulting from chestnuts crossed with cremellos.

b. Let C^B = chestnut and C^W = cremello. The parents and offspring of these crosses have the following genotypes: chestnut = C^BC^B; cremello = C^WC^W; palomino = C^BC^W.

Cross	Offspring
$C^BC^W \times C^BC^W$	13 C^BC^W, 6 C^BC^B, 5 C^WC^W
$C^BC^B \times C^BC^B$	16 C^BC^B
$C^WC^W \times C^WC^W$	13 C^WC^W
$C^BC^W \times C^BC^B$	8 C^BC^W, 9 C^BC^B
$C^BC^W \times C^WC^W$	11 C^BC^W, 11 C^WC^W
$C^BC^B \times C^WC^W$	23 C^BC^W

15. 0.15, or 15%.

17.

a. The 2 : 1 ratio in the progeny of the two spotted hamsters suggests lethality, and the 1 : 1 ratio in the progeny of a spotted hamster and a hamster without spots indicates that spotting is a heterozygous phenotype.

b. Because spotting is a heterozygous phenotype, obtaining Chinese hamsters that breed true for spotting is impossible.

19.

a. $^3/_4$ red, $^1/_4$ purple;

b. 1/2 red, 1/4 purple, 1/4 green;

c. 3/4 red, 1/4 purple;

d. 1/2 purple, 1/2 green;

e. 1/2 red, 1/2 purple.

24. The child's genotype has an allele for blood type B and an allele for blood type N that could not have come from the mother and must have come from the father. Therefore, the child's father must have an allele for type B and an allele for type N. George, Claude, and Henry are eliminated as possible fathers because they lack an allele for either type B or type N.

25.

a. All walnut (*Rr Pp*);

b. 1/4 walnut (*Rr Pp*), 1/4 rose (*Rr pp*), 1/4 pea (*rr Pp*), 1/4 single (*rr pp*);

c. 9/16 walnut (*R_ P_*), 3/16 rose (*R_ pp*), 3/16 pea (*rr P_*), 1/16 single (*rr pp*);

d. 3/4 rose (*R_ pp*), 1/4 single (*rr pp*);

e. 1/4 walnut (*Rr Pp*), 1/4 rose (*Rr pp*), 1/4 pea (*rr Pp*), 1/4 single (*rr pp*);

f. 1/2 rose (*Rr pp*), 1/2 single (*rr pp*)

26.

a. Brown backcross fish are *Bb Rr* blue fish are *Bb rr* red fish are *bb Rr* and white fish are *bb rr*.

b. $\chi^2 = 0.5$, df = 3; $0.9 < P < 0.975$.

We cannot reject the hypothesis and therefore assume that the backcross progeny appear in a 1 : 1 : 1 : 1 ratio.

c. All progeny would be *bb Rr*, or red fish.

d. F_1 will be all brown: *Bb Rr*.

Backcross progeny will be 1/2 brown and 1/2 red.

29.

a. Labrador retrievers vary in two loci, *B* and *E*. Black dogs have dominant alleles at both loci (*B_ E_*), brown dogs have *bb E_*, and yellow dogs have *B_ ee* or *bb ee*. Because all the puppies were black, all of them must have inherited a dominant *B* allele from the yellow parent and a dominant *E* allele from the brown parent. The brown female parent must have been *bb EE*, and the yellow male must have been *BB ee*. All the black puppies were *Bb Ee*.

b. Mating two yellow Labradors will produce yellow puppies only. Mating two brown Labradors will produce either brown puppies only, if at least one of the parents is homozygous *EE*, or $^{3}/_{4}$ brown and $^{1}/_{4}$ yellow puppies, if both parents are heterozygous *Ee*.

31. Let *A* and *B* represent the two loci. The F_1 heterozygotes are *Aa Bb*. The F_2 are *A_ B_* disc shaped, *A_ bb* spherical, *aa B_* spherical, *aa bb* long.

33.

a. According to the information in **Table 5.3**, Irish setters are *BB ee SS* and A^s or a^t. The *B* permits expression of black pigment, but the *ee* genotype prevents black color on the body coat, resulting in a reddish color except on the nose and in the eyes. The *S* prevents spotting, resulting in a uniform coat color.

b. Poodles are *SS*. Because the dominant *S* allele prevents spotting, no puppies from matings with poodles will have spotting.

c. St. Bernards are $a^y a^y$ *BB*, and Dobermans are $a^t a^t$ *EE SS*. The offspring will be genotype $a^y a^t$ *B_ E_ S_*. Because a^y, specifying yellow, is dominant over a^t, and the *E* allele allows expression of the *A* genotype throughout, the offspring will have yellow coats.

d. Rottweilers are $a^t a^t$ *BB EE SS*, and Labrador retrievers are $A^s A^s$ *SS*. The offspring will be $A^s a^t$ *B_ E_ SS*. The combination of the dominant A^s and *E* alleles should create solid coats.

35. Among females, $^{3}/_{4}$ hornless, $^{1}/_{4}$ horned.

39.

a. False. Genetic maternal effect means that the phenotype of the individual is determined solely by the genotype of the individual's mother. We know Martha's mother must have been *ss* because Martha is sinistral. If Martha was produced as a result of self-fertilization, then Martha must indeed be *ss*. But if Martha was produced by cross-fertilization, then we cannot know Martha's genotype without more information.

b. True. Martha's mother is *ss*, so Martha must be either s^+s or *ss*.

c. False. Because we do not know Martha's genotype, we cannot yet predict the phenotype of her offspring.

d. False. If Martha is s^+s, then all of her offspring will be dextral. If Martha is *ss*, then all of her offspring will be sinistral.

e. False. Martha's mother's phenotype is determined by the genotype of her mother (Martha's maternal grandmother). We know that Martha's mother's genotype must have been *ss*, so her mother's mother had at least one *s* allele. But we cannot know if she was a heterozygote or homozygous *ss*.

f. True. Because Martha's mother must have been *ss*, all her progeny must be sinistral.

45. Accounting for the phenocopies, we have 50% (subtracting the $^1/_3$ that are phenocopies from the 75%) of the puppies with the autosomal dominant genotype for long ears and 50% with the recessive genotype. Therefore, one parent is homozygous recessive and the other parent is a heterozygote.

Chapter 6

10.

1. A person may be aware of a genetic disease or risk factor in his or her family.
2. An older woman may be pregnant or contemplating pregnancy and may need information about risks and options for prenatal genetic testing.
3. A person may have tested positive for a genetic disease or risk factor and may need help interpreting the test results.
4. A person or couple may have a child with a genetic disease or may be caregivers for a person with a genetic disease, and require counseling on treatment options and management of the disease.

12. Amniocentesis is relatively safe, but results are not available until the 17th or 18th week of pregnancy. Chorionic villus sampling has a slightly higher risk of complications, including fetal injury, but results are available several weeks earlier.

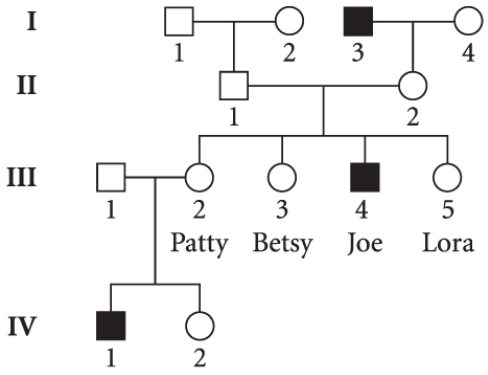
15. Direct-to-consumer genetic tests are tests purchased by consumers without an order from a medical professional. They may test for genetic conditions or predispositions, paternity, or ancestry. There are concerns that the tests may not be accurate and that the people who buy them may not be equipped to properly interpret or understand the information they provide. There is also a concern about the privacy or confidentiality of the individual ordering the test.

16. GINA prohibits discrimination by employers or health insurance companies based on information obtained by genetic testing. Requiring an individual to undergo genetic testing for employment or health insurance is also prohibited.

18. Study of human genetics is necessary to understand and overcome human genetic diseases. Because of humans' long life span, relatively large body size, and unique lifestyle and behaviors, animal models are nonexistent or insufficient for many genetic disorders. The careful preservation of marriage, birth, death, and health records in many societies provides a wealth of data for genetic analysis. The completion of the Human Genome Project now facilitates mapping and identification of human genes. Humans have a strong sense of identity and worth as individuals, and they wish to understand how an individual's genetic profile contributes to that person's health, behavior, abilities and disabilities, and individual future prospects. The study of human genetics can also reveal the historical origins and anthropological relationships of individuals and populations.

19.

a.



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Description
In the first generation, mating between two couples is shown. In the first couple, both male and female are unaffected and produce a son in second generation. In the second couple, the male is affected and female is unaffected and they produce a daughter in the second generation. The offspring in the second generation mate with each other and produce four offspring in the third generation; three unaffected females and one affected son. One of the unaffected females in the third generation mate with an unaffected male and produce an affected son and an unaffected daughter in the fourth generation.

b. X-linked recessive;

c. zero;

d. $^1/_4$;

e. $^1/_4$.

22.

a. Only males have the disease, it skips generations, and unaffected female carriers have both affected and unaffected sons. These observations are consistent with a recessive X-linked trait. Y-linked traits are transmitted directly from father to son and do not skip generations.

b. We will use X^+ to denote the normal X allele and X^d to denote the Dent allele.

I. 1 is X^+Y; 2 is X^+X^d.

II. 1 and 5 are X^dY, 7 and 8 are X^+X^d, and the rest do not have the disease allele.

III. 2 and 3 are carriers with X^+X^d, 4–7 are X^dY, and the rest do not have the disease allele.

IV. 2 is X^dY; 1 is X^+Y.

25.

a. Autosomal dominant. Affected males pass the trait to both sons and daughters. It does not skip generations, all affected individuals have affected parents, and it is extremely unlikely that multiple unrelated individuals mating into the pedigree are carriers of a rare trait.

b. X-linked dominant. Affected females pass the trait to sons and daughters, but affected males pass the trait to all daughters and no sons.

c. Y-linked. Affects only males and is passed from a father to all sons.

d. X-linked recessive or sex-limited autosomal dominant. We can eliminate Y linkage, because affected males do not pass the trait to their sons. X-linked recessive inheritance is consistent with more males than females affected, no father-to-son transmission, unaffected female carriers producing both affected and unaffected sons, and affected males producing unaffected female carriers. Sex-limited autosomal dominant inheritance is also consistent with unaffected heterozygous females producing affected heterozygous sons, unaffected homozygous recessive sons, and unaffected heterozygous or homozygous recessive daughters. We could distinguish between these two possibilities if we had enough data to determine whether affected males have both affected and unaffected sons, as would be expected from autosomal dominant inheritance, or whether affected males have only unaffected sons, as expected from X-linked recessive inheritance, but the number of offspring is too small to determine this.

e. Autosomal recessive. Unaffected parents produce affected progeny. The affected daughter must have inherited recessive alleles from both unaffected parents, so the trait must be autosomal. If it were X linked, her father would show the trait.

32. Migraine headaches: genetic and environmental. Markedly greater concordance in monozygotic twins, who are 100% genetically identical, than in dizygotic twins, who are 50% genetically identical, is indicative of a genetic influence. However, only 60% concordance for monozygotic twins indicates that environmental factors also play a role.

Eye color: genetic. Concordance is greater in monozygotic twins than in dizygotic twins. Monozygotic twins have 100% concordance, indicating that environment has no detectable

influence.

Measles: no detectable genetic influence. No difference in concordance between monozygotic and dizygotic twins. Some environmental influence can be detected, because monozygotic twins show less than 100% concordance.

Club foot: genetic and environmental. Markedly greater concordance in monozygotic twins indicates genetic influence. A strong environmental influence is indicated by the high discordance in monozygotic twins.

High blood pressure: genetic and environmental. Markedly greater concordance in monozygotic twins indicates genetic influence. Less than 100% concordance in monozygotic twins indicates environmental influence.

Handedness: no genetic influence. The concordance is the same in monozygotic and dizygotic twins, indicating no genetic influence. Less than 100% concordance in monozygotic twins indicates environmental influence. Tuberculosis: no genetic influence. Concordance is the same in monozygotic and dizygotic twins. The importance of environmental influence is indicated by the very low concordance in monozygotic twins.

35. The data suggest that schizophrenia has a strong genetic component. The biological parents of adoptees with schizophrenia are far more likely to have schizophrenia than are their adoptive parents, to whom they are genetically unrelated, despite the fact that the adoptees share their environment with their adoptive parents.

Chapter 7

15.

a. 1/4 wild-type eyes, wild-type wings; 1/4 red eyes, wild-type wings; 1/4 wild-type eyes, white-banded wings; and 1/4 red eyes, white-banded wings.

b. The F_1 heterozygotes inherited a chromosome with alleles for red eyes and white-banded wings (*re wb*) from one parent and a chromosome with alleles for wild-type eyes and wild-type wings (re^+ wb^+) from the other parent. These are, therefore, the phenotypes of the nonrecombinant progeny, present in the highest numbers. The recombinants are the 19 with red eyes, wild-type wings and the 16 with wild-type eyes, white-banded wings.

$$\begin{aligned}\text{recombination frequency} &= \frac{\text{number of recombinant progeny}}{\text{total number of progeny}} \times 100\% \\ &= (19 + 16)/879 \times 100\% \\ &= 4.0\%\end{aligned}$$

The distance between the genes is 4 map units.

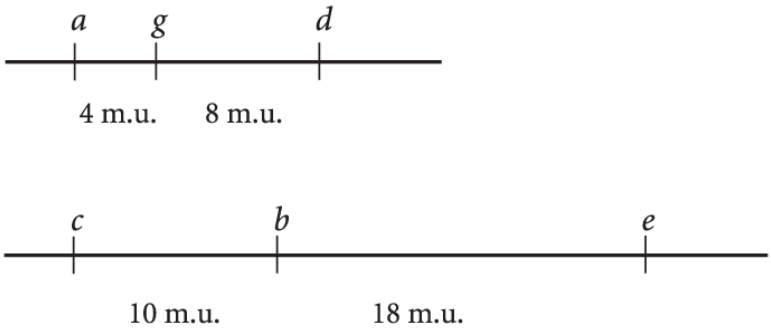
17. The genes are linked and have not assorted independently.

18.

Heart-shaped, numerous spines	33.7%
Normal, few spines	33.7%
Heart-shaped, few spines	16.3%
Normal, numerous spines	16.3%

22. The distances between the genes are indicated by the recombination rates. Because loci *R* and L_2 have the highest recombination rate, they must be the farthest apart, and W_2 is in the middle. The order of the genes is *R*, W_2, L_2.

28.



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Description
From left to right, the D N A is labeled small A, 4 m u, small G, 8 m u, and small D in the first line drawing. In the second line drawing, from left to right the D N A is labeled small C, 10 m u, small B, 18 m u, and small E.

Gene *f* is unlinked to either of these groups; it is on a third linkage group.

31.

a. All the progeny receive $\underline{p \quad sh\text{-}1 \quad Hb^2}$ from the male parent, shown as the lower chromosome in each progeny type. The upper chromosomes are the products of meiosis in the heterozygous parent and are identified in the table. The nonrecombinants have $\underline{p \quad sh\text{-}1 \quad Hb^2}$ or $\underline{P \quad Sh\text{-}1 \quad Hb^1}$; the double crossovers have $\underline{p \quad Sh\text{-}1 \quad Hb^2}$ or $\underline{P \quad sh\text{-}1 \quad Hb^1}$. The two classes differ in the *Sh*-1 locus; therefore, *Sh*-1 is the middle locus.

b. *P* and *Sh*-1: recombinants have $\underline{P \quad sh\text{-}1}$ or $\underline{p \quad Sh\text{-}1}$.
RF = (57 + 45 + 1)/708 = 0.145 = 14.5 m.u.

Sh-1 and *Hb:* recombinants have $\underline{Sh\text{-}1 \quad Hb^2}$ or $\underline{sh\text{-}1 \quad Hb^1}$.
RF = (6 + 5 + 1)/708 = 0.017 = 1.7 m.u.

c. Expected double crossovers = recombination frequency 1 × recombination frequency 2 × total progeny = 0.145 × 0.017 × 708 = 1.7

Coefficient of coincidence = observed double crossovers/expected double crossovers = 1/1.7 = 0.59

Interference = 1 - coefficient of coincidence = 0.41

32.

a. The gene order is *Wx, V, Sh*

b. *Wx* – *V* distance is 7%, or 7 m.u.;

sh – *V* distance is 30%, or 30 m.u.

c. Coefficient of coincidence = 0.80

Interference = 0.20

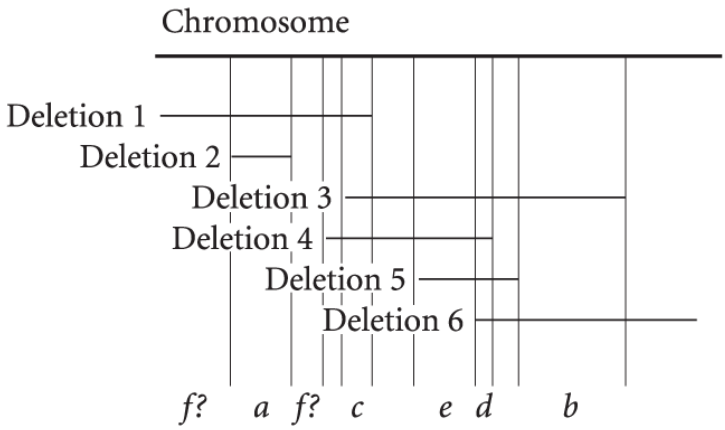
38. This lod score indicates that the probability of observing this degree of association if the marker is linked to asthma is 100 times higher than if the marker has no linkage to asthma. A lod score of 3, for a ratio of 1000, is generally considered convincing evidence of linkage.

40. Enzyme 1 is located on chromosome 9. Chromosome 9 is the only chromosome that is present in the cell lines that produce enzyme 1 and absent in the cell lines that do not produce enzyme 1.

Enzyme 2 is located on chromosome 4. Chromosome 4 is the only chromosome that is present in cell lines that produce enzyme 2 (C and D) and absent in cell lines that do not produce enzyme 2 (A and B).

Enzyme 3 is located on the X chromosome. The X chromosome is the only chromosome present in the three cell lines that produce enzyme 3 and absent in the cell line that does not produce enzyme 3.

41.



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Description

The map shows six deletions, which includes a horizontal line that represents a chromosome at the top and 6 more horizontal lines below representing deletions labeled 1 through 6. Each deletion is of different length. There are 10 vertical lines drawn downward from the chromosome, with irregular intervals in between. The gap is highest between ninth and tenth line and is lowest between the third, fourth, seventh, and eighth vertical line. Deletion 1 is from the starting of the chromosome till vertical line 5. Deletion 2 is between vertical lines 1 and 2. Deletion 3 is between vertical lines 4 and 10. Deletion 4 is between vertical lines 3 and 8. Deletion 5 is between vertical lines 6 and 9. Deletion 6 is from the starting of vertical lines 7 till the end of the chromosome. The map also shows the location of recessive mutations as follows:

f question mark: Between starting of chromosome and vertical line 1.

a: Between vertical lines 1 and 2.

f question mark: Between vertical lines 2 and 3.

c: Between vertical lines 4 and 5.

e: Between vertical lines 6 and 7.

d: Between vertical lines 7 and 8.

b: Between vertical lines 9 and 10.

The location of *f* is ambiguous; it could be in either location shown in the deletion map.

Chapter 8

19.

a. Duplications;

b. polyploidy;

c. deletions;

d. inversions;

e. translocations.

20.

a. Tandem duplication of AB;

b. displaced duplication of AB;

c. paracentric inversion of DEF;

d. deletion of B;

e. deletion of FG;

f. paracentric inversion of CDE;

g. pericentric inversion of ABC;

h. duplication and inversion of DEF;

i. duplication of CDEF, inversion of EF.

23.

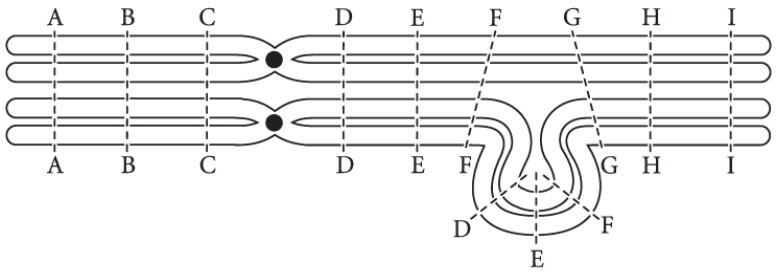
a. $^1/_3$ white-eyed Notch females, $^1/_3$ wild-type females, and $^1/_3$ wild-type males

b. $^1/_3$ red-eyed Notch females, $^1/_3$ wild-type females, and $^1/_3$ white-eyed males

c. $^1/_3$ white-eyed Notch females, $^1/_3$ white-eyed females, and $^1/_3$ white-eyed males

25.

a.



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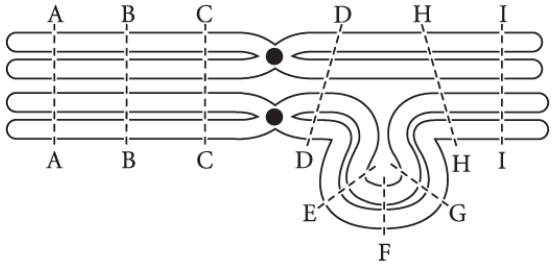
Description

The illustration shows two X-shaped horizontally placed chromosomes. The chromosomes have one long and one short arm. The upper chromosome has no loop and has the genes as follows: big A, big B, big C, in the first arms, then big D, big E, big F, big G, big H, and big I in the second arms. The chromosome below has an additional looped out region on the long arm with genes big D through big F.

b.



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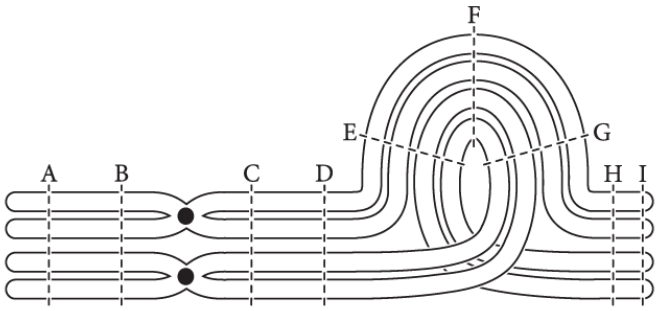
Description

The illustration shows two X-shaped horizontally placed chromosomes. The chromosomes have one long and one short arm. The upper chromosome has no loop and has the genes as follows: big A, big B, big C, in the first arms, then big D, big H, and big I in the second arms. The chromosome below has an additional looped out region on the long arm with genes big E, big F, and big G between big D and big H.

c.



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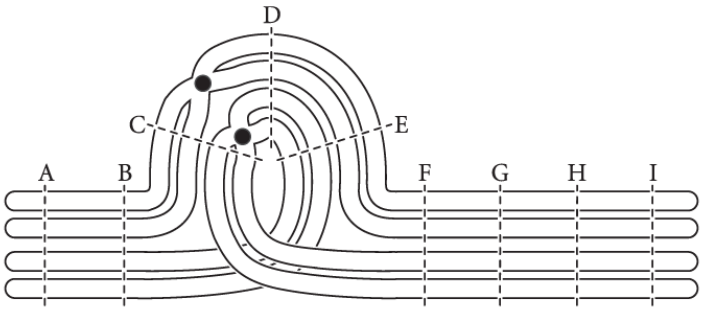
Description

The illustration shows two X-shaped horizontally placed chromosomes with loops. The chromosomes have one long and one short arm. Both the chromosomes have the following genes: big A, big B, in the first arms, then big C, big D, big E, big F, big G, big H, and big I in the second arms of the top chromosome, and big C, big D, big G, big F, big E, big H, and big I in the bottom chromosome. The looped out region is formed with genes big E, big F, and big G in the long arms.

d.



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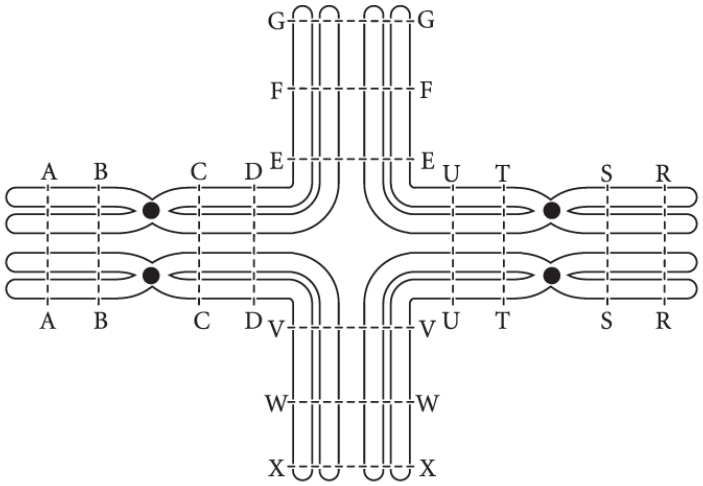
Description
The illustration shows two X-shaped horizontally placed chromosomes with loops. The chromosomes have one long and one short arm. The top chromosome has the following genes: big A, big B, big C, in the first arms, then big D, big E, big F, big G, big H, and big I in the second arms. The bottom chromosome has the following genes: big A, big B, big E, big D, in the first arms, then big C, big F, big G, big H, and big I in the second arms. The looped out region is formed with genes big C, centromere, big D, and big E.

27. If crossing over occurs within the pericentric inversion at a rate of 26% of meioses, then 13% of the woman's oocytes will have duplication or deficient chromosome 8, and the probability of the couple having a child with a syndrome caused by the crossing over is 13%.

28.

a.



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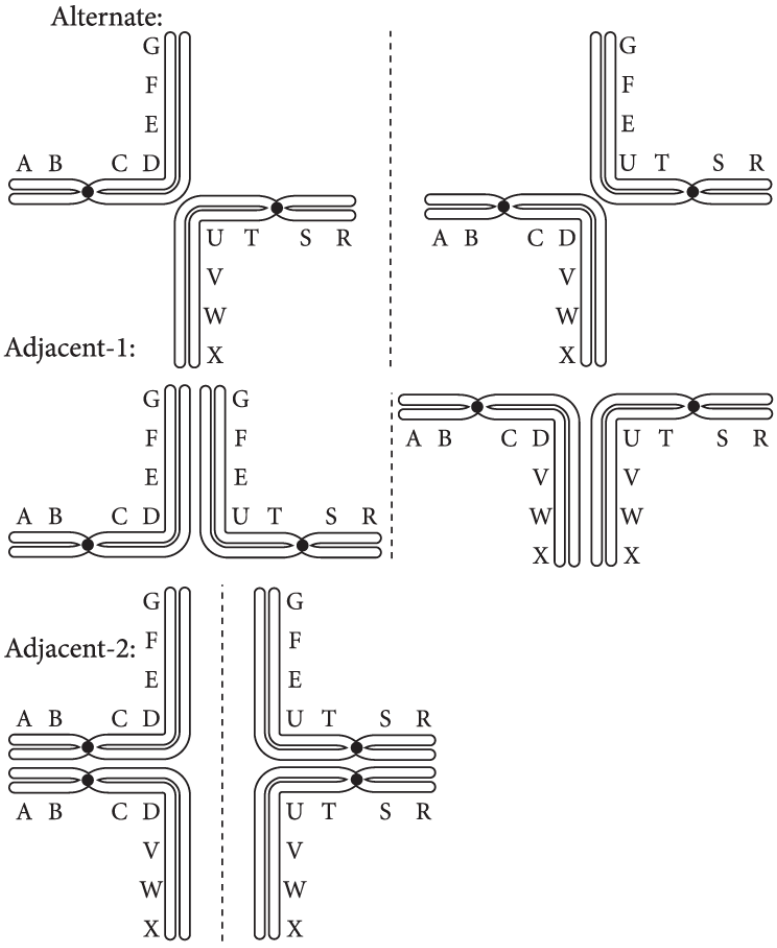
Description

The diagram shows four X chromosomes in the shape of an uppercase L. One side of this chromosome is aligned with a chromosome with genes big A, big B, in the first arms, then big C, big D placed horizontally and big E, big F, big G going upward, and the other side is aligned with a chromosome with genes big R, big S, in the first arms, then big T, big U (placed horizontally from right to left), and big E, big F, and big G (moving upwards). The below chromosome forms the same type of structure inverted with genes big V, big W, and big X moving downward in place of big E, big F, and big G. The position of each chromosome results in the shape of a cross.

b.



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Description

"Adjacent 1" shows four structures. The first structure shows one chromosome with genes big A, big B, in the first arms, then big C, big D big E, big F, and big G in the second arms runs horizontally from left to right then upwards from big D. The second chromosome with genes big R, big S, in the first arms, then big T, big U, big V, big W, and big X in the seconds arms runs horizontally from right to left and moves downward at big U. The second structure is similar to the first one, but the first chromosome goes downward and the second chromosome goes upward. There is dotted vertical line in between these two structures. The third structure shows both the chromosomes moving upward after moving rightward and leftward respectively. The upward genes are big E, big F, and big G on both chromosomes. The fourth structure shows both the chromosomes moving downward after moving rightward and leftward respectively. The downward genes are big V, big W, and big X on both chromosomes. There is a dotted vertical line in between these third and fourth structures. "Adjacent 2" shows four X chromosomes. One side has a chromosome with genes, from left to right, big A, big B, in the first arms, then big C, big D placed horizontally and big E, big F, big G going upward from big D, and the vertically mirrored side is a chromosome with genes, from right to left, big R, big S, in the first arms, then big T, big U, and big E, big F, and big G going upwards from big U. The below chromosomes form the same type of structure inverted with genes big V, big W, and big X moving downward in place of big E, big F, and big G. The position of each chromosome results in the shape of a cross. A dotted vertical line runs through the center of the chromosomes dividing it symmetrically into two equal halves.

c. Alternate: Gametes contain either both normal or both translocation chromosomes and are all viable.

$$\underline{\mathrm{AB \cdot CDEFG}} + \underline{\mathrm{RS \cdot TUVWX}}$$

and

$$\underline{\mathrm{AB \cdot CDVWX}} + \underline{\mathrm{RS \bullet TUEFG}}$$

Adjacent-1: Gametes contain one normal and one translocation chromosome, resulting in the duplication of some genes and a deficiency of others.

$$\underline{\mathrm{AB \cdot CDEFG}} + \underline{\mathrm{RS \cdot TUEFG}}$$

and

$$\underline{\mathrm{AB \cdot CDVWX}} + \underline{\mathrm{RS \cdot TUVWX}}$$

Adjacent-2 (rare): Gametes contain one normal and one translocation chromosome, with the duplication of some genes and a deficiency of others.

$$\underline{\mathrm{AB \bullet CDEFG}} + \underline{\mathrm{AB \bullet CDVWX}}$$

and

$$\underline{\mathrm{RS \bullet TUVWX}} + \underline{\mathrm{RS \bullet TUEFG}}$$

29. The nondisjunction event took place during meiosis II of the egg.

33. The high incidence of Down syndrome in Bill's family and among Bill's relatives is consistent with familial Down syndrome, caused by a Robertsonian translocation of chromosome 21. Bill and his sister, who are unaffected, are unaffected carriers of the translocation and have 45 chromosomes. Their children and Bill's brother, who have Down syndrome, have 46 chromosomes. From the information given, there is no reason to suspect that Bill's wife, Betty, has any chromosome abnormalities. Therefore, statement *d* is most likely correct. All other statements are incorrect.

a. Incorrect. Bill is an unaffected carrier of the translocation and has 45 chromosomes.

b. Incorrect. There is no reason to suspect that Bill's wife, Betty, has any chromosome abnormalities.

c. Incorrect. Bill and Betty's children have familial Down syndrome and have 46 chromosomes.

d. Correct. Bill's sister is an unaffected carrier of the translocation and has 45 chromosomes.

e. Incorrect. Bill is an unaffected carrier of the translocation and has 45 chromosomes.

f. Incorrect. There is no reason to suspect that Bill's wife, Betty, has any chromosome abnormalities.

g. Incorrect. Bill's brother has familial Down syndrome and has 46 chromosomes.

35.

a. Possible gamete types:

i. normal chromosome 13 and normal chromosome 22;

ii. translocated chromosome 13 + 22;

iii. translocated chromosome 13 + 22 and normal chromosome 22;

iv. normal chromosome 13;

v. normal chromosome 13 and translocated chromosome 13 + 22;

vi. normal chromosome 22.

b. Zygote types:

i. 13, 13, 22, 22; normal;

ii. 13, 13 + 22, 22; translocation carrier;

iii. 3, 13 + 22, 22, 22; trisomy 22;

iv. 13, 13, 22; monosomy 22;

v. 13, 13, 13 + 22, 22; trisomy 13;

vi. 13, 22, 22; monosomy 13.

c. 50%.

39.

a. Such allotriploids could have 1*n* from species I and 2*n* from species II for 3*n* = 18; alternatively, they could have 2*n* from species I and 1*n* from species II for 3*n* = 15.

b. 4*n* = 28.

c. 2*n* + 1 = 9.

d. 2*n* + 1 = 13.

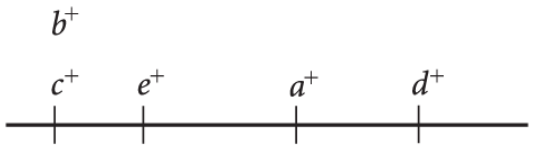
e. 2*n* + 2 = 10.

f. Allotetraploids must have chromosomes from both species and total 4*n*. There are three possible combinations for such allotetraploids: 2*n* from each: 2(4) + 2(7) = 22; 1*n* from species I + 3*n* from species II: 1(4) + 3(7) = 25; and 3*n* from species I + 1*n* from species II: 3(4) + 1(7) = 19.

Chapter 9

18. Isolate DNA from water samples taken from the two streams. Sequence the DNA, and identify the bacterial species based on differences in their DNA.

23. Only genes located near one another on the bacterial chromosome will be cotransformed. However, by performing transformation experiments and screening for different pairs of cotransformed genes, bacterial genes can be mapped. Gene pairs that are never cotransformed must be relatively far apart on the chromosome, whereas gene pairs that are cotransformed are more closely linked. From the data, we see that gene a^+ is cotransformed with both e^+ and d^+. However, genes d^+ and e^+ are not cotransformed, indicating that a^+ and e^+ are more closely linked than d^+ and e^+. Gene a^+ is not cotransformed with either b^+ or c^+, yet gene e^+ is. This observation indicates that gene e^+ is more closely linked to genes b^+ and c^+ than is gene a^+. The orientation of genes b^+ and c^+ relative to e^+ cannot be determined from the data provided.



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Description
The genes are as follows: small C superscript positive, small E superscript positive, small A superscript positive, and small D superscript positive. Small B superscript positive is written on top left of the chromosome, above small C superscript positive.

28. The original infecting phages were wild type ($a^+\ b^+$) and doubly mutant ($a^-\ b^-$). The $a^+\ b^-$ and $a^-\ b^+$ plaques are produced by recombinant phages.

The frequency of recombination is the total number of recombinant plaques divided by the total number of plaques: 677/4854 = 0.14.

29.

a. Transductants were initially screened for the presence of *proC*$^+$. Thus, only *proC*$^+$ transductants were identified.

b. The wild-type genotypes (*proC*$^+$ *proA*$^+$ *proB*$^+$ *proD*$^+$) represent single transductants of *proC*$^+$. Both the *proC*$^+$ *proA*$^-$ *proB*$^+$ *proD*$^+$ and *proC*$^+$ *proA*$^+$ *proB*$^-$ *proD*$^+$ genotypes represent cotransductants of *proC*$^+$, *proA*$^-$ and *proC*$^+$, *proB*$^-$.

c. Both *proA* and *proB* were cotransduced with *proC* at about the same rate, which suggests that they are similar in distance from *proC*. The *proD* gene was never cotransduced with *proC*, suggesting that *proD* is more distant from *proC* than *proA* and *proB*. However, there were a small number of all types of cotransductants, and the absence of cotransduction between *proD* and *proC* might be due to chance.

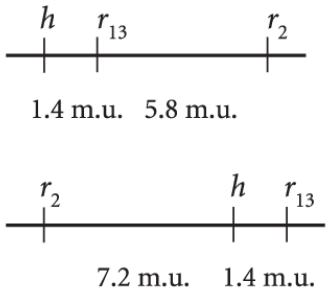
30.

Plaque phenotype	Expected number
$c^+ m^+$	460 (nonrecombinant)
$c^- m^-$	460 (nonrecombinant)
$c^+ m^-$	40 (recombinant)
$c^- m^+$	40 (recombinant)
Total plaques	1000

32.

a. The recombination frequency between r_2 and h is $^{13}/_{180} = 0.072$, or 7.2%. The recombination frequency between r_{13} and h is $^{3}/_{217} = 0.014$, or 1.4%.

b.



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Description
From left to right, the D N A is labeled small H, 1 point 4 m u, small R subscript 13, 5 point 8 m u, and small R subscript 2 in the first line drawing. From left to right, the D N A is labeled small R subscript 2, 7 point 2 m u, small H, 1 point 4 m u, and small R subscript 13 in the second line drawing.

33.

a.

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Description
In line drawing, from left to right, the D N A is labeled small H superscript positive, small S small T superscript positive, and small C superscript positive.

b.

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Description
In the line drawing, from left to right, the D N A is labeled small H superscript positive, 7 point 1 m u, small S small T superscript positive, 24 point 1 m u, and small C superscript positive.

c. $$\text{Coefficient of coincidence} = \frac{(6+5)}{(0.071 \times 0.241 \times 942)}$$
$$= 0.68.$$

Interference = 1 − 0.68 = 0.32.

Chapter 10

18. Took X-ray diffraction pictures used in constructing the structure of DNA: **(i)** Franklin and Wilkins

Determined that DNA contains nitrogenous bases:

a. Kossel

Identified DNA as the genetic material in bacteriophages:

f. Hershey and Chase

Discovered regularity in the ratios of different bases in DNA:

j. Chargaff

Determined that DNA is responsible for transformation in bacteria:

g. Avery, MacLeod, and McCarty

Worked out the helical structure of DNA by building models:

c. Watson and Crick

Discovered that DNA consists of repeating nucleotides:

d. Levene

Determined that DNA is acidic and high in phosphorus:

e. Miescher

Conducted experiments showing that RNA can serve as the genetic material in some viruses:

b. Fraenkel-Conrat

Demonstrated that heat-killed material from bacteria can genetically transform live bacteria:

h. Griffith

19. No, the student has not demonstrated that transformation has taken place. A single mutation could convert the IIR strain into the virulent IIS strain. Thus, the student cannot

determine whether the conversion from IIR to IIS is due to transformation or to a mutation. Additionally, the student has not demonstrated that the heat was sufficient to kill all the IIS bacteria. A second useful control experiment would have been to inject the heat-killed IIS into mice and see if any of the IIS bacteria survived the heat treatment.

24. Tubes 1, 4, and 5. The DNA of the bacteriophage contains phosphorus and the protein contains sulfur. When the bacteriophages infect a cell, they inject their DNA into the cell, but the protein coats stay on the surface of the cell. The phage protein coats are sheared off in the blender, whereas the bacterial cells containing the phage DNA form a pellet at the bottom of the tube. Thus, tubes containing bacteria infected with ^{35}S-labeled bacteriophages will have radioactivity associated with the protein coats, whereas tubes containing bacteria infected with ^{32}P-labeled bacteriophages will have radioactivity associated with the cells.

26. The phosphate backbone of DNA molecules typically carries a negative charge, thus making the DNA molecules attractive to the positive pole of the current.

27. Approximately 5×10^{20} nucleotide pairs. Stretched end to end, the DNA would reach 1.7×10^{8} km.

29.

a.

Organism and tissue	(A + G)/(T + C)	(A + T)/(C + G)
Sheep thymus	1.03	1.36
Pig liver	0.99	1.44
Human thymus	1.03	1.52
Rat bone marrow	1.02	1.36
Hen erythrocytes	0.97	1.38
Yeast	1.00	1.80
E. coli	1.04	1.00
Human sperm	1.00	1.67
Salmon sperm	1.02	1.43

Herring sperm	1.04	1.29

b. The (A + G)/(T + C) ratio of about 1.0 is constant for these organisms. Each of them has a double-stranded genome. The percentage of purines should equal the percentage of pyrimidines in double-stranded DNA, which means that (A + G) = (T + C). The (A + T)/(C + G) ratios are not constant. The number of A − T base pairs relative to the number of C − G base pairs is unique to each organism and can vary among the different organisms.

c. The (A + G)/(T + C) ratio is about the same for the three sperm samples. Although the sperm is haploid, its DNA is still double stranded. As in part *b*, the percentage of purines should equal the percentage of pyrimidines.

32. Adenine = 15%; guanine = 35%; cytosine = 35%.

36. Virus I is a double-stranded RNA virus. Uracil is present, indicating an RNA genome. As expected for a double-stranded genome, the amounts of adenine and uracil are equal, as are the amounts of guanine and cytosine.

Virus II is a double-stranded DNA virus. The presence of thymine indicates that the viral genome is DNA. As expected for a double-stranded DNA molecule, the amounts of adenine and thymine are equal, as are the amounts of guanine and cytosine.

Virus III is a single-stranded DNA virus. The presence of thymine indicates a DNA genome. However, the amounts of thymine and adenine are unequal, as are the amounts of guanine and cytosine, which suggests a single-stranded DNA molecule.

Virus IV is a single-stranded RNA virus. The presence of uracil indicates an RNA genome. However, the amount of adenine does not equal that of uracil, and the amount of guanine does not equal that of cytosine, which suggests a single-stranded genome.

37. 100,000

38.

a. 1. Neither $5'$ carbon of the two sugars is directly linked to phosphorus.

2. Neither $5'$ carbon of the two sugars has a hydroxyl group attached.

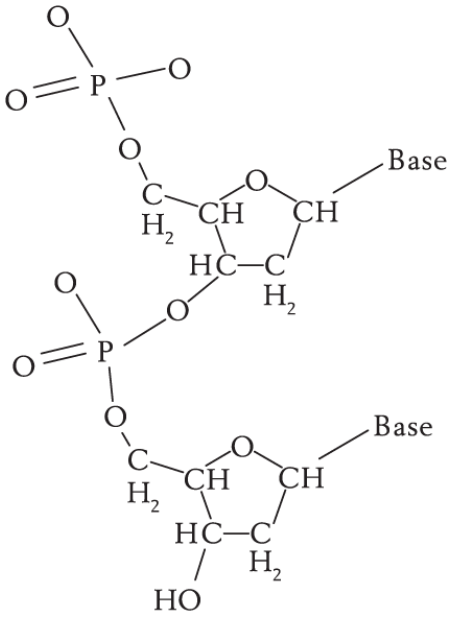
3. Neither sugar molecule has oxygen in its ring structure between the $1'$ and $4'$ carbons.

4. In both sugars, the $2'$ carbon has a hydroxyl group attached, which does not occur in deoxyribonucleotides.

5. At the $3'$ position in both sugars, only hydrogen is attached, as opposed to a hydroxyl group.

6. The $1'$ carbon of both sugars has a hydroxyl group, as opposed to just a hydrogen attached.

b.



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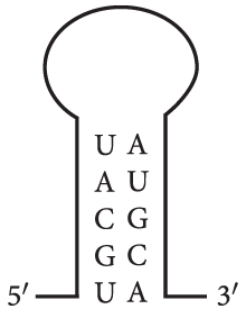
Description
The structure begins with a phosphate group that has a central P atom, with 3 O atoms; one of which is double bonded to P. The P with 3 oxygen atoms is bound to a sugar molecule through the O atom on 5 prime C atom attached to C H 2 C H on the pentose sugar. The sugar has O atom on top and clockwise the positions are occupied by C H attached to a base, C H 2, C H, and C H C H 2 O. The 4 prime C is attached to another phosphate group, which is likewise attached to a pentose sugar with base at 2 prime C. This sugar has C H O H at 4 prime.

40. Hairpins often consist of a stem and a loop. The stem consists of inverted complementary RNA sequences, and the loop consists of a region of noncomplementary sequences. The inverted complements form the stem structure, and the loop of the hairpin is formed by the noncomplementary sequences.

$$5'-\text{UGCAU}-3' \ldots \text{ unpaired nucleotides } \ldots 5'-\text{AUGCA}-3'$$



Description
The R N A starts with 5 prime, goes vertically upward as U G C A U forms a loop and comes downward forming complementary base pairing as A U G C A and ends at 3 prime.

41. The genetic material will contain complex information, replicate faithfully, and encode the phenotype. Even if the genetic material on the planet is not DNA, it must have these properties. Additionally, the genetic material will be stable.

Chapter 11

18. Bacterial chromosomes are usually circular, whereas eukaryotic chromosomes are linear. A single bacterial chromosome generally contains the entire genome, whereas the eukaryotic genome is divided into multiple chromosomes. Bacterial chromosomes are generally much smaller than eukaryotic chromosomes and have only a single origin of DNA replication, whereas eukaryotic chromosomes contain multiple origins of DNA replication. Eukaryotic chromosomes contain DNA packaged into nucleosomes; nucleosomes are absent from bacterial chromosomes (although both bacterial and archaeal DNA is complexed with proteins). The condensation state of eukaryotic chromosomes varies with the cell cycle.

19. Because each nucleosome contains two molecules of H2A and only one molecule of H1, eukaryotic cells will have more H2A than H1. Because each nucleosome contains two molecules of H2A and two molecules of H3, eukaryotic cells should have equal amounts of these two histones.

21.

a. None;

b. embryonic;

c. adult;

d. none.

22.

a. 3.2×10^7;

b. 2.9×10^8.

23. More acetylation. Regions of DNase I sensitivity are less condensed than DNA that is not sensitive to DNase I; the sensitive DNA is less tightly associated with nucleosomes, and it is in a more open state. Such a state is associated with the acetylation of lysine in the histone tails. Acetylation eliminates the positive charge of the lysine and reduces the affinity of the histone for the negatively charged phosphates of the DNA backbone.

27. The upper molecule, because it has a higher percentage of A–T base pairs, will have a lower melting temperature. A–T base pairs have two hydrogen bonds and are thus less stable than G–C base pairs, which have three hydrogen bonds.

29. If the mutation is located within a chloroplast gene, then it should be inherited only from the female parent. We would expect the following results, no matter which trait is dominant:

Wild-type male × light-green female → offspring all light green

Light-green male × wild-type female → offspring all wild type

If the mutation is in a nuclear gene, then both parents can pass the light-green mutation to their offspring. If the wild type is dominant, then we would expect the following results:

Wild-type male × light-green female → offspring all wild type

Light-green male × wild-type female → offspring all wild type

If the light-green phenotype is dominant, then we would expect the following results:

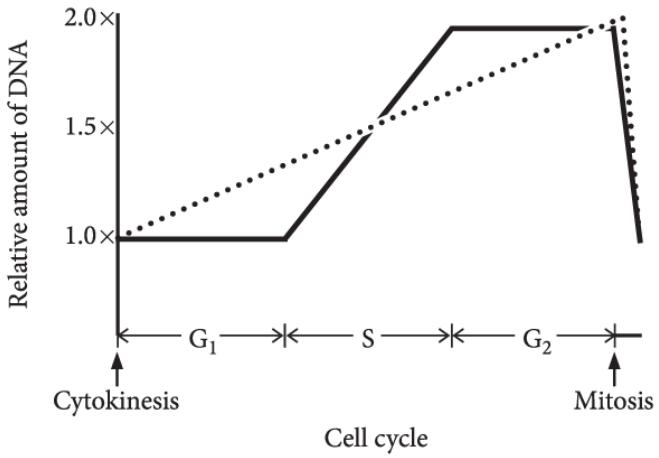
Wild-type male × light-green female → offspring all light green

Light-green male × wild-type female → offspring all light green

30. The pedigree indicates that the neurological disorder is a cytoplasmically inherited trait. Only females pass the trait to their offspring. The trait does not appear to be sex-specific in that males as well as females can have the disorder.

33. Maternal inheritance indicates that a *poky* mutation has a mitochondrial origin because mitochondrial genomes are inherited maternally in *Neurospora*. Biparental inheritance suggests that the *poky* mutation is of nuclear origin. In *Neurospora*, nuclear genes exhibit biparental inheritance.

34. Amounts of nuclear DNA should increase during only the S phase before declining at cytokinesis. Mitochondrial DNA levels should increase throughout the cell cycle before declining at cytokinesis.



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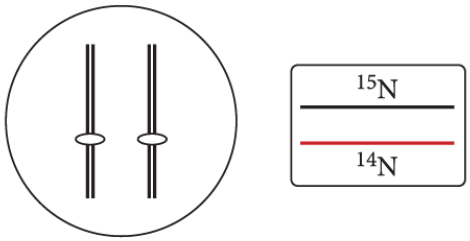
Description

The horizontal axis represents cell cycle and starts from cytokinesis at origin, then G 1, S, G 2, and mitosis. The vertical axis represents relative amount of D N A and has three markings as follows: times 1 point 0, times 1 point 5, and times 2 point 0. The graph shows a solid and a dotted line. The dotted line starts from (cytokinesis, times 1 point 0), rises till (mitosis, times 2 point 0), and declines after that. The solid line also starts from (cytokinesis, times 1 point 0), remains constant till the end of G 1, and then rises to reach (end of S, times 2 point 0), again becomes constant till (mitosis, times 2 point 0), and declines after that.

Chapter 12

21.

a. Cells in G_1, before switching to medium with ^{14}N



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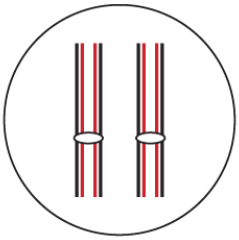
Description
A cell shows two submetacentric chromosomes. Outside the cell, a rectangular box is drawn which represents a black line as N 15 and a red line as N 14.

b. Cells in G_2, after switching to medium with ^{14}N



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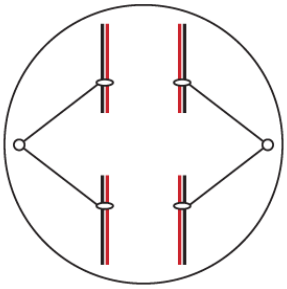
Description
The cell shows two submetacentric chromosomes. The chromosomes are represented by four parallel lines. Two inner lines are red, while the outer lines are black in each chromosome.

c. Cells in anaphase of mitosis, after switching to medium with ^{14}N



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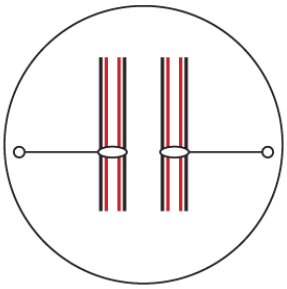
Description
The cell shows four chromatids; two on each side, having one red and one black line, attached to spindle fibers on their respective ends.

d. Cells in metaphase I of meiosis, after switching to medium with ^{14}N



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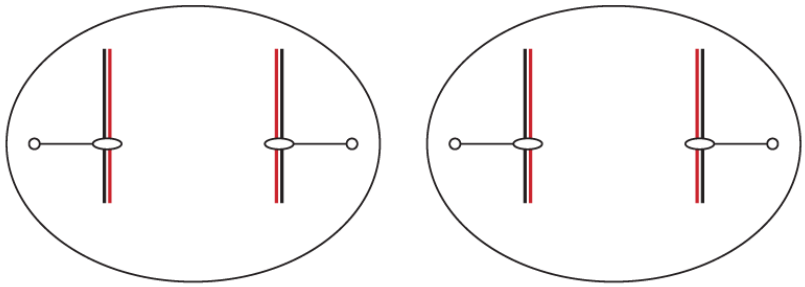
Description
The cell shows two submetacentric chromosomes. The chromosomes are represented by four parallel lines. Two inner lines are red, while the outer lines are black in each chromosome. The centromeres are attached to spindle fibers on their respective ends.

e. Cells in anaphase II of meiosis, after switching to medium with ^{14}N



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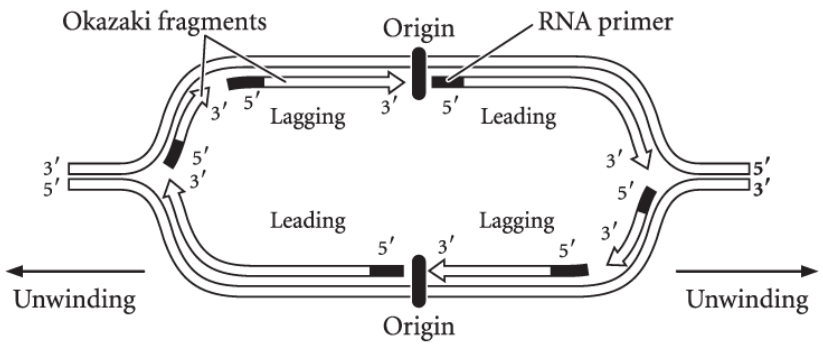
Description
Each cell shows two chromatids; one on each side, with one red and one black line, attached to spindle fibers through centromeres on their respective ends.

22. Theta replication, 5 minutes; rolling-circle replication, 10 minutes.

24.



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Description
The line drawing shows a double-stranded D N A with a replication bubble in between. The replication bubble has the newly synthesized strands within. The center of the strands is marked origin both on top and below. Below the top strand a newly synthesized strand is present in fragments called okazaki fragments on the left of the origin with its top left end, 5 prime, and the top right end, 3 prime labeled lagging strand. On the right of the origin is a continuous leading strand with its top left end, 5 prime, starting from the origin and right end, 3 prime, pointing towards the winded end of the D N A strand. Above the bottom strand, a newly synthesized strand has a continuous leading strand on the left of the origin and lagging strand on right of the origin. R N A primers are attached at the 5 prime ends of every fragment of the newly synthesized D N A. Two arrows below the double stranded D N A point outward from the center and are labeled unwinding.

26.

a. More errors in replication;

b. primers would not be removed;

c. primers that had been removed would not be replaced.

29. Primase is required for replication initiation in theta replication. If primase is nonfunctional, then replication initiation would not take place, and no replication would occur. Rolling-circle replication does not require primase. A single-strand break within one strand provides a 3′-OH group to which nucleotides can be added, so rolling-circle replication could occur without a functional primase.

31. Two distinct bands. If the original histones remained on one strand, then we would expect to see the octamers with original histones nearer the bottom of the centrifuge tube in a distinct band. Octamers with newly synthesized histones would be lighter and would appear in a distinct band higher in the tube.

33. The RNA component of telomerase is needed to provide a template for synthesizing the telomeric sequences at the ends of the chromosomes. A large deletion would probably prevent telomere synthesis.

38. Protein B may be needed for the successful initiation of replication at origins of replication. Protein B is present at the beginning of S phase but disappears by the end of it. Protein A may be responsible for removing or inactivating protein B. As levels of protein A increase, levels of protein B decrease, preventing extra initiation events. When protein A is mutated, it can no longer inactivate protein B; thus, successive rounds of replication can begin, owing to the high levels of protein B. When protein B is mutated, it cannot assist initiation, and replication ceases.

Chapter 13

15.

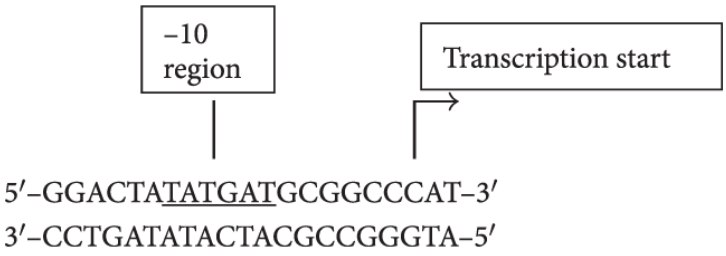
a. Single stranded. If it was double stranded, we would expect nearly equal percentages of adenine and uracil, as well as equal percentages of guanine and cytosine. In this RNA molecule, the percentages of these bases are not equal.

b. A = 42%, T = 23%, C = 14%, and G = 21%

16. 5′–AUAGGCGAUGCCA–3′

21. $\frac{T}{A}$GCAATT

24.



Description
The sequence of double-stranded D N A is as follows: First strand: 5 prime- G G A C T A T A T G A T G C G G C C C A T-3 prime; Second strand: 3 prime-C C T G A T A T A C T A C G C C G G G T A-5 prime. The region A T A T G A T in the first strand is marked negative 10 region and transcription starts from the last third nucleotide in the first strand.

The −10 consensus sequence, or Pribnow box, has the consensus sequence TATAAT. However, few bacterial promoters actually contain the exact consensus sequence. The transcription start site is about 10 bp downstream of the −10 consensus sequence.

25.

a. Would probably affect the −10 consensus sequence, which would most likely decrease transcription.

b. Would probably affect the −35 consensus sequence, reducing or inhibiting transcription.

c. Unlikely to have any effect on transcription.

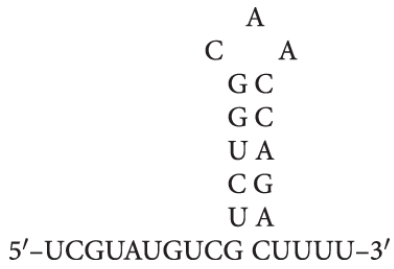
d. Would have little effect on transcription.

29.

a.

b. A rho-independent terminator.

c.



Description
The sequence is as follows: 5 prime-U C G U A U G U C G strand goes upward U C U G G forms a loop C A A comes downward forming complementary base pairing with the upward strand as C C A G A , and then continues horizontally as C U U U U-3 prime.

35. If TBP cannot bind to the TATA box, then genes with these promoters will be transcribed at very low levels or not at all.

39.

a. From experiment 3 and its corresponding lane in the gel, pTFIIB and the RNA polymerase from *S. pombe* (pPol II) appear to have been sufficient to determine the transcription start site. The *S. pombe* start site was used, even though many of the other transcription factors were from *S. cerevisiae*.

b. When TFIIE and TFIIH were individually exchanged, transcription was affected. However, the paired exchange of TFIIE and TFIIH allowed for transcription, suggesting that TFIIE and TFIIH undergo a species-specific interaction essential for transcription or that the absence of this interaction inhibits transcription. Similar results were observed in the paired exchange of TFIIB and RNA polymerase, which suggests that species-specific interaction between TFIIB and RNA polymerase is needed for transcription.

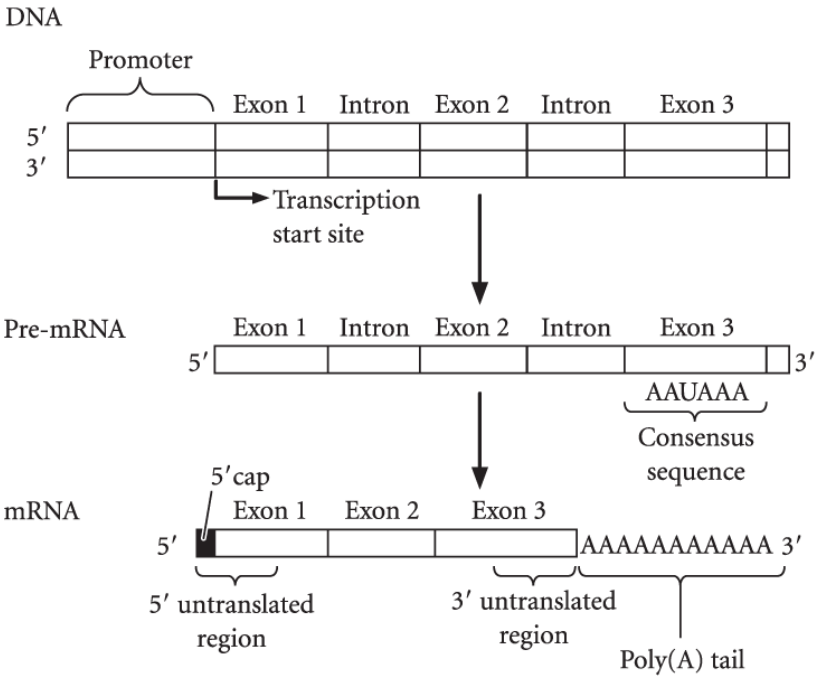
c. TFIIB probably interacts with both RNA polymerase and other general transcription factors necessary for initiation at the promoter. The data indicate that TFIIB and RNA polymerase II must be from the same species for transcription to take place. TFIIB

potentially assembles downstream of the TATA box but in association with other transcription factors located at or near the TATA box. Possibly, these downstream interactions between TFIIB and the other transcription factors stimulate conformational changes in RNA polymerase and the DNA sequence, enabling transcription to begin at the appropriate start site. In experiment 3, both the RNA polymerase and TFIIB were from *S. pombe*, leading to the positioning of the RNA polymerase at the *S. pombe* transcription start site by pTFIIB.

Chapter 14

22. The large size of the dystrophin gene is probably due to the presence of many intervening sequences, or introns, within the coding region of the gene.

28.



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Description
The illustration starts from a double-stranded D N A labeled 5 prime and 3 prime on the left end of first and second strand, respectively. The D N A is labeled for the following regions from left to right: promoter, exon 1, intron, exon 2, intron, and exon 3. At the end of promoter is the transcription start site. A downward arrow from the D N A leads to a single-stranded pre-m R N A labeled 5 prime to 3 prime on left and right ends, respectively. The pre-m R N A has the following sequence: exon 1, intron, exon 2, intron, and exon 3. Exon 3 has A A U A A A labeled consensus sequence. A downward arrow from the pre-m R N A leads to a single-stranded m R N A labeled 5 prime to 3 prime on left and right ends, respectively. On its 5 prime is 5 prime cap. It has the following regions: exon 1, exon 2, exon 3, and A A A A A A A A A A A A labeled poly A tail. 5 prime end has 5 prime untranslated region from 5 prime cap to half of exon 1. 3 prime end has 3 prime untranslated region from half of exon 3 till the end.

a. Lies upstream of the translation start codon. In bacteria, the ribosome-binding site, or Shine–Dalgarno sequence, is within the 5′ untranslated region. However, eukaryotic mRNA does not have an equivalent sequence, and a eukaryotic ribosome binds at the 5′ cap of the mRNA molecule.

b. A DNA sequence recognized and bound by the transcription apparatus to initiate transcription.

c. Lies downstream of the coding region of the gene; determines the location of the $3'$ cleavage site in the pre-mRNA molecule.

d. First nucleotide transcribed into RNA; located 25–30 nucleotides downstream of the TATA box.

e. A sequence of nucleotides located at the $3'$ end of the mRNA that is not translated into proteins. However, it does affect the stability and translation of the mRNA.

f. Noncoding DNA sequences that occur within coding regions of a gene; removed during processing of the pre-mRNA.

g. Coding regions of a gene.

h. A sequence of adenine nucleotides added to the $3'$ end of the pre-mRNA; affects mRNA stability and the binding of the ribosome to the mRNA.

i. Functions in binding of the ribosome to the mRNA and in mRNA stability.

33. The mutation will probably prevent $3'$ cleavage and addition of the poly(A) tail, resulting in more rapid degradation of the mRNA. Less of the encoded protein will be produced.

38.

a. In trans-splicing, exons from different genes are spliced together during RNA processing. As a result, the amino acid sequence of the translated protein is encoded by two or more different genes. According to the concept of colinearity, we would have expected the DNA sequence of a single gene to correspond directly to the amino acid sequence of the protein it encodes.

b. Different mature mRNAs can be produced from a single gene by alternative splicing. Thus, different proteins can be encoded within the same gene, as opposed to one gene corresponding to one protein, as is predicted by the concept of colinearity.

c. In RNA editing, genetic information is added to the pre-mRNA after it is transcribed; the mature mRNA therefore contains information that was not part of the DNA from which it was transcribed. As a result, the nucleotide sequence of the gene does not correspond to the amino acid sequence of the protein—a clear violation of the concept of colinearity.

Chapter 15

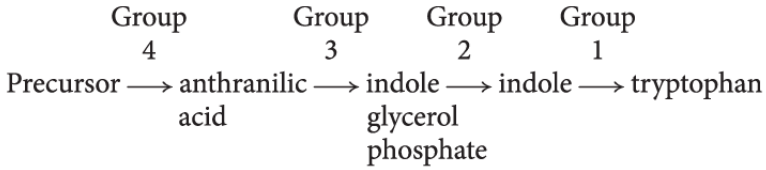
16. The mutations can be assembled into four groups:

Group 1 mutants (*trp-1*, *trp-10*, *trp-11*, *trp-9*, *trp-6*, and *trp-7*) can grow only on a minimal medium supplemented with tryptophan.

Group 2 mutants (*trp-3*) can grow on a minimal medium supplemented with either tryptophan or indole.

Group 3 mutants (*trp-2* and *trp-4*) can grow on a minimal medium supplemented with tryptophan, indole, or indole glycerol phosphate.

Group 4 mutants (*trp-8*) can grow on a minimal medium supplemented with the addition of tryptophan, indole, indole glycerol phosphate, or anthranilic acid.



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Description
The flowchart is as follows: Precursor, forward arrow, anthranilic acid, forward arrow, indole glycerol phosphate, forward arrow, indole, forward arrow, and tryptophan. From left to right the forward arrows are labeled from group 4 through group 1.

20.

a. 1;

b. 2;

c. 3;

d. 3;

e. 4.

21. 3^3, or 27.

22.

a. amino–fMet-Phe-Lys-Phe-Lys-Phe–carboxyl

b. amino–fMet-Tyr-Ile-Tyr-Ile–carboxyl

c. amino–fMet-Asp-Glu-Arg-Phe-Leu-Ala–carboxyl

d. amino–fMet-Gly–carboxyl (The stop codon UAG follows the codon for glycine.)

26.

a. 3′–CCG–5′ or 3′–UCG–5′

b. 3′–UUC–5′

c. 3′–AUU–5′ or 3′–UUU–5′ or 3′–CUU–5′

d. 3′–ACC–5′ or 3′–GCC–5′

e. 3′–GUC–5′

30. initiation factor 3

fMet-tRNA$_i^{fMet}$

30S initiation complex

70S initiation complex

elongation factor Tu

elongation factor G

release factor 1

33.

a. 3′–UGC–5′;

b. threonine.

34.

a. The lack of IF-3 would prevent protein synthesis. IF-3 separates the large and small ribosomal subunits, which is required for the initiation of translation. The absence of IF-3 would mean that translation would not be initiated and no proteins would be synthesized.

b. No translation would take place. IF-2 is necessary for the initiation of translation. The lack of IF-2 would prevent fMet-$tRNA_i^{fMet}$ from being delivered to the small ribosomal subunit, thus blocking translation.

c. Although translation would be initiated by the delivery of fMet-$tRNA_i^{fMet}$ to the ribosome–mRNA complex, no other amino acids would be delivered to the ribosome. EF-Tu, which binds to GTP and the charged tRNA, is necessary for elongation. This three-part complex enters the A site of the ribosome. If EF-Tu were not present, the charged tRNA would not enter the A site, and translation would stop.

d. EF-G is necessary for the translocation (movement) of the ribosome along the mRNA in the $5' \rightarrow 3'$ direction. Once a peptide bond had formed between Met and Pro, the lack of EF-G would prevent the movement of the ribosome along the mRNA, so no new codons would be read. The formation of the dipeptide Met-Pro does not require EF-G.

e. The release factors RF-1 and RF-2 recognize the stop codons and bind to the ribosome at the A site. They then interact with RF-3 to promote cleavage of the peptide from the tRNA at the P site. The absence of the release factors would prevent the termination of translation at the stop codon.

f. ATP is required for tRNAs to be charged with amino acids by aminoacyl-tRNA synthetases. Without ATP, the charging would not take place, and no amino acids would be available for protein synthesis.

g. GTP is required for the initiation, elongation, and termination of translation. If GTP were absent, protein synthesis would not take place.

39. NMD should not be a problem. NMD is thought to be dependent on exon-junction proteins that are normally removed from the mRNA by the movement of the ribosomes during translation but trigger NMD when they are not removed. If the first ribosome to read the mRNA inserts an amino acid for the stop codon due to the action of PTC124, then it should not stall at the stop codon and should remove any exon-junction proteins. The result is that the mRNA will be stabilized and protected from NMD, thus allowing translation to continue.

42.

a. These results suggest that the initiator tRNA plays a role in selection of the start codon. In eukaryotes, the ribosome attaches to the $5'$ cap and then scans the mRNA until it locates the AUG start codon. When the anticodon on the $tRNA_i^{Met}$ was mutated, it appears that a

different start codon was selected because protein synthesis occurred, but the resulting protein had more or fewer amino acids than normal.

b. The initiation of translation in bacteria occurs in a different way—it requires that the 16S RNA of the small ribosomal subunit interact with the Shine–Dalgarno sequence. This interaction lines up the ribosome over the initiation codon. If the anticodon of $tRNA_i^{fMet}$ were mutated, no initiator tRNA would pair with the initiation codon, and no protein synthesis would take place.

c. When the anticodon of the $tRNA_i^{Met}$ is mutated to $5'$–CCA–$3'$, initiation takes place at the first $5'$–UGG–$3'$ codon (complementary to $5'$–CCA–$3'$) encountered by the scanning ribosome. In some mRNAs, this codon will occur before the normal $5'$–AUG–$3'$ start codon, so extra amino acids will be added to the protein; in other mRNAs, it will occur after the normal start codon, resulting in a protein with fewer amino acids.

Chapter 16

12.

a. Inactive repressor;

b. active repressor.

15. RNA polymerase will bind the *lac* promoter poorly, significantly decreasing the transcription of the *lac* structural genes.

21.

	Lactose absent		Lactose present	
Genotype of strain	**β-Galactosidase**	**Permease**	**β-Galactosidase**	**Permease**
$lacI^+\ lacP^+\ lacO^+\ lacZ^+\ lacY^+$	−	−	+	+
$lacI^-\ lacP^+\ lacO^+\ lacZ^+\ lacY^+$	+	+	+	+
$lacI^+\ lacP^+\ lac^{Oc}\ lacZ^+\ lacY^+$	+	+	+	+
$lacI^-\ lacP^+\ lacO^+\ lacZ^+\ lacY^-$	+	−	+	−
$lacI^-\ lacP^-\ lacO^+\ lacZ^+\ lacY^+$	−	−	−	−
$lacI^+\ lacP^+\ lacO^+\ lacZ^-\ lacY^+/lacI^-\ lacP^+\ lacO^+\ lacZ^+\ lacY^-$	−	−	+	+
$lacI^-\ lacP^+\ lac^{Oc}\ lacZ^+\ lacY^+/lacI^+\ lacP^+\ lacO^+\ lacZ^-\ lacY^-$	+	+	+	+
$lacI^-\ lacP^+\ lacO^+\ lacZ^+\ lacY^-/lacI^+\ lacP^-\ lacO^+\ lacZ^-\ lacY^+$	−	−	+	−
$lacI^+\ lacP^-\ lac^{Oc}\ lacZ^-\ lacY^+/lacI^-\ lacP^+\ lacO^+\ lacZ^+\ lacY^-$	−	−	+	−
$lacI^+\ lacP^+\ lacO^+\ lacZ^+\ lacY^+/lacI^+\ lacP^+\ lacO^+\ lacZ^+\ lacY^+$	−	−	+	+
$lac^{Is}\ lacP^+\ lacO^+\ lacZ^+\ lacY^-/lacI^+\ lacP^+\ lacO^+\ lacZ^-\ lacY^+$	−	−	−	−
$lacI^S\ lacP^-\ lacO^+\ lacZ^-\ lacY^+/\ lacI^+\ lacP^+\ lacO^+\ lacZ^+\ lacY^+$	−	−	−	−

23. The *lacI* gene encodes the *lac* repressor protein, which can diffuse within the cell and attach to any operator. It can therefore affect the expression of genes on the same molecule or on a different molecule of DNA. The *lacO* gene encodes the operator. It affects the binding of RNA polymerase to DNA and therefore affects the expression of genes only on the same molecule of DNA.

29.

a. No gene expression;

b. transcription of the structural genes only when alanine levels are low;

c. no transcription;

d. no transcription;

e. transcription will proceed;

f. transcription will proceed;

g. transcription will proceed.

32. To block transcription, you need to disrupt the action of RNA polymerase either directly or indirectly. Antisense RNA containing sequences complementary to the gene's promoter should inhibit the binding of RNA polymerase. If transcription initiation requires the assistance of an activator protein, then antisense RNA complementary to the activator protein–binding site of the gene could also disrupt transcription.

Chapter 17

19. It is likely that flowering will not occur. The protein encoded by *FLD* is a deacetylase enzyme that removes acetyl groups from histones surrounding the flowering locus *C* (*FLC*). Once the acetyl groups are removed, the chromatin structure within this region is restored, which inhibits transcription from the *FLC* locus. *FLC* encodes a transcription factor whose expression activates other genes that suppress flowering. If *FLC* transcription is active, then flowering will not occur.

20. DNA methylation is associated with transcriptional repression. If X31b is taken up by rapidly dividing cancer cells, it could stimulate methylation of DNA sequences, leading to transcriptional repression of genes. The repression of transcription could slow the growth of the cancer cells.

24. The action of an enhancer on the promoter of a gene is blocked when the insulator is located between the enhancer and the promoter. It is likely that transcription of genes A, B, and C will be stimulated by the enhancer, because there is no insulator between these genes and the enhancer. Transcription of gene D will not be stimulated, because the insulator is located between gene D and the enhancer.

25. The fruit flies will develop

a. male characteristics;

b. male characteristics;

c. both male and female characteristics.

27. The presence of AU-rich elements is associated with rapid degradation of mRNA molecules that contain them through RNA interference. If the AU-rich element were deleted, then miRNA would not be able to bind to it, and mRNA degradation would not be initiated. It is likely that this mRNA molecule would be more stable, resulting in increased expression of the protein encoded by the mRNA.

Chapter 18

19.

a. Leucine, serine, or phenylalanine;

b. isoleucine, tyrosine, leucine, valine, or cysteine;

c. phenylalanine, proline, serine, or leucine;

d. methionine, phenylalanine, valine, arginine, tryptophan, leucine, isoleucine, tyrosine, histidine, or glutamine, or a stop codon could result as well.

23.

a. A single-base-pair substitution resulting in a missense mutation.

b. A single-base-pair substitution resulting in a nonsense mutation.

c. The deletion of a single nucleotide resulting in a frameshift mutation.

d. A six-base-pair deletion resulting in the elimination of two amino acids (Arg and Leu) from the protein.

e. The insertion of three nucleotides resulting in the addition of a Leu codon.

24. Four of the six Arg codons could be mutated by a single-base-pair substitution to produce a Ser codon. All of these codons could undergo a second mutation at a different site to produce the amino acid Arg.

Original Arg codon	Ser codon	Restored Arg codon
CGU	**A**GU	AG**G** or AG**A**
CGC	**A**GC	AG**G** or AG**A**
AGA	AG**U**	**C**GU
	AG**C**	**C**GC
AGG	AG**C**	**C**GC
	AG**U**	**C**GU

26. No, hydroxylamine cannot reverse nonsense mutations. Hydroxylamine modifies cytosine-containing nucleotides and can result only in C • G → T • A transition mutations. In a stop codon, the C • G → T • A transition will result only in a different stop codon.

28.

a. The strand contains two purines, adenine and guanine. Because repair of depurination typically results in adenine being substituted for the missing purine, only the loss of the guanine by depurination will result in a mutant sequence.

5′–A**G**–3′	to	5′–A**A**–3′
3′–T**C**–5′		3′–T**T**–5′

b. Deamination of guanine, cytosine, and adenine can occur. However, the deamination of only cytosine and adenine are likely to result in mutant sequences, because the deamination products can form improper base pairs. Deaminated guanine does not pair with thymine but can still form two hydrogen bonds with cytosine; thus, no change will occur.

5′–A**G**–3′	if A is deaminated, then	5′–**G**G–3′
3′–**T**C–5′		3′–**C**C–5′
5′–A**G**–3′	if C is deaminated, then	5′–A**A**–3′
3′–T**C**–5′		3′–T**T**–5′

30. PFI1 causes transitions, PFI2 causes transversions or large deletions, PFI3 causes transitions, and PFI4 causes single-base insertions or deletions.

34. The flanking repeat is in boldface type.

a. 5′–ATTCGAAC**TGAC**[transposable element]**TGAC**CGATCA–3′

b. 5′–ATT**CGAA**[transposable element]**CGAA**CTGACCGATCA–3′

37. The pairs of sequences in **(b)** and**(d)** are inverted repeats because they are both reversed and complementary and might be found at the ends of insertion sequences.

39. These results could be explained by hybrid dysgenesis, with strain B harboring *P* elements and strain A having no *P* elements.

43. The appearance of purple spots of varying sizes in these few yellow corn kernels could be explained by transposition. The yellow kernels may be due to inactivation of a pigment gene by insertion of a *Ds* element in the plant bearing this ear. Because the *Ds* element cannot transpose on its own, the mutant allele is stable in the absence of *Ac*, and the plant produces yellow kernels when fertilized by pollen from the same strain (lacking *Ac*). However, a few kernels may have been fertilized by pollen from a different strain with an active *Ac* element. The *Ac* element could then mobilize transposition of the *Ds* element out of the pigment gene, restoring the pigment gene's function. Excision of the *Ds* element earlier in kernel development would produce larger clones of cells producing purple pigment. Excision later in kernel development would produce smaller clones of purple cells.

45. The breeder can look for plants that have increased levels of mutation in either their germline or somatic tissues. Potentially mutant plants may have been exposed to standard mutagens that damage DNA. If they are defective in DNA repair, they should have higher rates of mutation.

Chapter 19

29. *Ara*I

31. 10

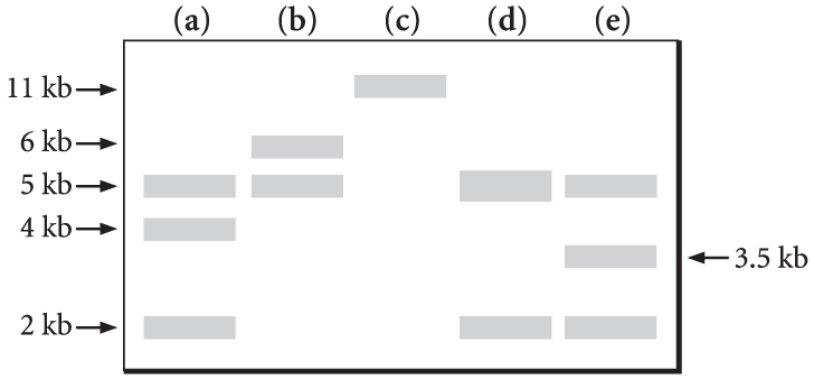
34.

a. 460,800;

b. 1,036,800;

c. 5,120,000.

35.



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Description
A scale on the gel shows 8 labels 12, 9, 7, 5, 4, 3, 2, and 1 from top to bottom. In the gel below bands are shown for each of the three lanes represented by a shaded rectangle. Lane “H p a 1” has bands at 9, 3, and 1 positions. Lane “Hind 3” has bands at 12, 7, and 3 positions. Lane “both” has bands at 7, 5, 4, 3, 2, and 1 positions.

39.

a. Plasmid;

b. phage λ

c. cosmid;

d. bacterial artificial chromosome.

43. A cDNA library, created from mRNA isolated from the venom gland. Bacteria cannot splice introns, so a cDNA sequence that has been reverse transcribed from mRNA, and therefore has no intron sequences, is needed. The venom gland should be the source of the mRNA used for cDNA synthesis because that is where the toxin gene is likely to be transcribed and where abundant mRNA for the toxin is produced.

44.

a. Val-Tyr-Lys-Ala-Lys-Trp;

b. 128.

46. 5′–GCATCAGTA–3′

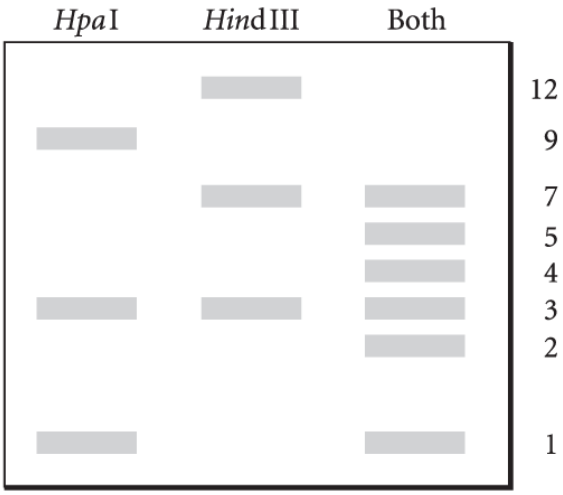
52.

a. The figure shows that both larvae and adults whose parents were injected with dsRNA for *unc22A* express high levels of GFP protein. However, larvae and adults whose parents were injected with dsRNA for *gfp* do not express GFP. These results indicate that dsRNA specifically inhibits expression of the gene corresponding to the dsRNA but not unrelated genes.

b. Injection of dsRNA corresponding to introns and promoter sequences would have little effect on *gfp* gene expression because RNA interference works by targeting mRNA. Introns and promoter sequences are not present in mRNA.

Chapter 20

25.



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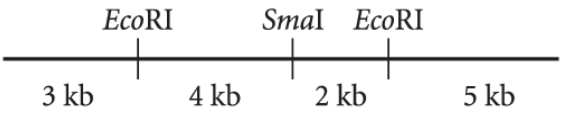
Description
A scale on the gel shows 6 markings labeled 11 k b, 6 k b, 5 k b, 4 k b, 3 point 5 k b, and 2 k b fom top to bottom. In the gel below bands are shown for each of the five lanes represented by a shaded rectangle. Lane “a” has bands at 5 k b, 4 k b, and 2 k b. Lane “b” has bands at 6 and 5 k b. Lane “c” has only one band at 11 k b. Lane “d” has bands at 5 and 2 k b. Lane “e” has bands at lines 5, 3 point 5, and 2 k b.

26.



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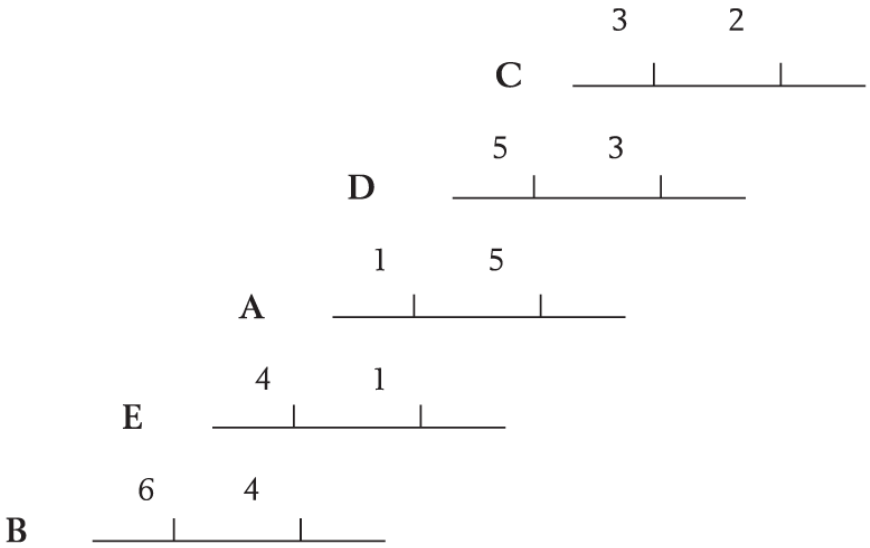
Description
From left to right, the D N A is labeled 3 k b, Eco R 1, 4 k b, S m a 1, 2 k b, Eco R 1, and 5 k b.

27.



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Description
Line drawing “B” shows markings on the line labeled 6 and 4. Line drawing “E” shows markings on the line labeled 4 and 1. Line drawing “A” shows markings on the line labeled 1 and 5. Line drawing “D” shows markings on the line labeled 5 and 3. Line drawing “C” shows markings on the line labeled 3 and 2.

30.

a. Chromosome 22 has the highest density and greatest number of genes, whereas the Y chromosome has the lowest density and fewest genes.

b. The known genes found in this region (0 – 1, 000, 000 bp) are *PLCXD1*, *GTPBP6*, *PPP2R3B*, and *SHOX*.

36. Paralogs, because both evolved from duplication of a primordial β-globin gene.

37. Genes 2 and 24 are expressed at far higher levels in the antibiotic-resistant bacteria than in the nonresistant cells. Conversely, genes 4, 17, and 22 are downregulated. All of these genes may be involved in antibiotic resistance. The upregulated genes may be involved in metabolism of the antibiotic or may perform functions that are inhibited by the antibiotic. The downregulated genes may be involved in uptake of the antibiotic or represent a cellular mechanism that accentuates the potency of the antibiotic. Characterization of these genes might lead to information regarding the mechanism of antibiotic resistance and thus to the design of new antibiotics that can circumvent this resistance mechanism.

45.

a. Genome size: 0.5 million to over 9 million base pairs in prokaryotes; 12 million (yeast) to hundreds of billions in eukaryotes.

b. Number of genes: from 500 to 8000 in prokaryotes; from 6000 to 32,000 in eukaryotes.

c. Gene density (bp/gene): approximately 1000 bp/gene in prokaryotes; varies from 2000 bp/gene to greater than 100,000 bp/gene in eukaryotes.

d. Number of exons: with few exceptions, prokaryotic genes have zero or only one exon; multicellular eukaryotic genes typically have multiple exons.

52.

a. The minimal genome required for life might be determined by examining simple free-living organisms with small genomes to determine which genes they share. Mutations could then be systematically induced to determine which of the shared genes are essential for these organisms to survive. Genes could then be deleted one by one. Elimination of any of the essential genes would result in loss of viability. Alternatively, essential genes could be assembled through synthetic biology, creating entirely novel organisms with different sets of genes. The ability of these synthetic organisms to survive and reproduce could then be evaluated to determine which genes are essential.

b. Among the social and ethical concerns would be the ethics of creating entirely new organisms and the question of whether such novel synthetic organisms could or would be used to develop pathogens for biological warfare or terrorism. There would also be uncertainty about the new organisms' effects on ecosystems if they escaped or were released.

Chapter 21

2. An epigenetic trait is one that is stable and passed on to descendant cells or offspring but does not involve changes in the DNA base sequence. Many epigenetic traits are caused by changes in gene expression resulting from modifications to chromatin. Although stable, many epigenetic traits can be influenced by environmental factors.

23. All female bees would develop characteristics of queens, regardless of whether they were fed royal jelly.

24. No. At CpG dinucleotides, two cytosine nucleotides sit diagonally across from each other on the two DNA strands, and both are methylated. The presence of 5-methylcytosine on both strands is required for maintaining methylation after replication. Following replication, one strand of each new DNA molecule is methylated, and one is not. Special methyltransferase enzymes recognize the methyl group on one strand and methylate the cytosine on the other strand, perpetuating the methylated state of the DNA. Individual cytosine nucleotides will not have a nucleotide on the opposite strand that can be methylated following replication. Therefore, no new methyl groups will be added by the methyltransferases after replication.

29.

a. Both X chromosomes would be active.

b. Both X chromosomes would be active.

31. We would expect to see differences in DNA methylation and histone acetylation that alter the expression of genes involved in responses to stress. We would also expect that, as adults, the rats would show increased fear and heightened hormonal responses to stress.

32. Because only the original pregnant females were exposed to vinclozolin, the effects on the sperm of $F_2 - F_4$ mice cannot be explained by direct effects of vinclozolin on male fertility. Because of the high frequency (90%) of the mice affected in $F_2 - F_4$, it appears unlikely that the effects are due to mutations induced by vinclozolin. These transgenerational effects are most likely due to epigenetic changes. This conclusion is supported by the different DNA methylation patterns of the $F_1 - F_4$ offspring of vinclozolin-exposed females. DNA methylation is known to affect chromatin structure and is responsible for some epigenetic effects.

Chapter 22

14.

a. The products of *bicoid* and *dorsal* affect embryonic polarity by regulating the transcription of target genes.

b. The product of *nanos* regulates the translation of *hunchback* mRNA.

20.

a. Higher levels of maternal *bicoid* mRNA in the anterior cytoplasm of eggs. After fertilization, embryos would have higher levels of Bicoid protein and thus enlarged anterior and thoracic structures.

b. Lower levels of Bicoid protein and embryos with small head structures.

22. Egg-polarity (genetic maternal effect): *bicoid*, *nanos*. Gap: *hunchback*, *Krüppel*. Pair-rule: *even-skipped*, *fushi tarazu*. Segment-polarity: *gooseberry*. Homeotic: *labial* (*lab*), *Antennapedia* (*Ant*), *Ultrabithorax* (*Ubx*), *abdominal A* (*abdA*).

25. A plant that lacked both class A and class B genes would express only class C genes in all four whorls, resulting in flowers with only carpels. A plant that lacked both class B and class C genes would express only class A genes in all four whorls and would result in flowers with only sepals.

28. Widespread expression of *shh* in the eye primordium of blind cavefish causes degeneration of lens cells. In the F_1 progeny of blind cavefish and surface fish, the expression of *shh* is intermediate between that in blind cavefish and that in surface fish, resulting in small eyes.

29. TET enzymes normally demethylate DNA, so mutants defective in TET will have increased DNA methylation. Increased methylation represses genes involved in eye development, leading to decreased eye size in zebrafish.

30. 2600

Chapter 23

17. Deletions can cause the loss of one or more tumor-suppressor genes. Inversions and translocations can inactivate tumor-suppressor genes if the chromosome breaks that accompany them lie within tumor-suppressor genes. A translocation can also place a proto-oncogene in a new location, where it is activated by different regulatory sequences, causing the overexpression or unregulated expression of the proto-oncogene. Inversions and translocations can also bring parts of two different genes together, causing the synthesis of a novel fusion protein that is oncogenic.

20. After its integration into a host genome, a retrovirus promoter can drive the overexpression of a cellular proto-oncogene. Alternatively, the integration of a retrovirus can inactivate a tumor-suppressor gene. A few retroviruses carry oncogenes that are altered versions of host proto-oncogenes. Other viruses, such as human papillomavirus, express gene products (proteins or RNA molecules) that interfere with host-cell cycle regulation by inactivating tumor-suppressor proteins.

23. If the differences in cancer rates are due to genetic differences in the two populations, then people who migrated from Utah or Shanghai to other locations would have rates of prostate cancer incidence similar to those of people who stayed in Utah or Shanghai. Moreover, different ethnic groups in Utah or Shanghai would have different rates of cancer. If the cancer rates are due to environmental factors, then people who migrated from Utah or Shanghai would have rates of prostate cancer determined by their new locations and not by their place of origin, and people from different ethnic groups living in the same location would have similar rates of cancer.

24. Oncogenes are mutated copies of genes that normally encode substances that stimulate cell division, whereas tumor-suppressor genes encode substances that inhibit cell division. *TP53* encodes a protein that ensures that cell division takes place only under appropriate conditions, so it normally acts as a brake on cell division. When the *TP53* gene is mutated, cell division is unregulated and abnormal, and tumors develop. Thus, *TP53* is a tumor-suppressor gene, in spite of the fact that it is usually inherited as a dominant mutation (usually associated with oncogenes).

31. The deleted region contains a tumor-suppressor gene. Tumor suppressors act as inhibitors of cell proliferation. The deletion of tumor-suppressor genes therefore permits the uncontrolled cell proliferation that is characteristic of cancer. Oncogenes, on the other hand, function as stimulators of cell division. Deletion of oncogenes therefore prevents cell proliferation and usually cannot cause cancer.

Chapter 24

16.

a. A discontinuous characteristic, because only a few distinct phenotypes are present, and the characteristic is determined by alleles at a single locus.

b. A discontinuous characteristic, because there are only two phenotypes (dwarf and normal), and a single locus determines the characteristic.

c. A quantitative characteristic, because susceptibility is a continuous trait determined by multiple genes and environmental factors (an example of a quantitative phenotype with a threshold effect).

d. A quantitative characteristic, because it is determined by many loci (an example of a meristic characteristic).

e. A discontinuous characteristic, because only a few distinct phenotypes are determined by alleles at a single locus.

18.

a. All weigh 10 grams.

b. $^{1}/_{16}$ weigh 16 grams, $^{4}/_{16}$ weigh 13 grams, $^{6}/_{16}$ weigh 10 grams, $^{4}/_{16}$ weigh 7 grams, and $^{1}/_{16}$ weigh 4 grams.

20. That six or more loci take part.

24. The sum of the weights is 676; dividing by 10 students yields a mean of 67.6 kg.

The variance is $s^2 = \frac{\sum (x_i - x)^2}{n-1} = 2024.4/9 = 224.9$

The standard deviation $= s = \sqrt{s^2} = 15$

26. The correlation coefficient *r* is calculated from the formula

$$r = \frac{\mathrm{COV}_{xy}}{s_x s_y}$$
$$\mathrm{cov}_{xy} = 72$$
$$s_x = 40.33$$
$$s_y = 1.87$$
$$r = \frac{72}{(40.33 \times 1.87)} = 0.95$$

27.

a. R = 3.99/[(2.2)(2.1)] = 0.88;

b. 67.8 inches.

28.

a. 0.38;

b. 0.69.

33.

a. 0.75.

b. Its inaccuracy might be due to a difference between the environmental variance of the genetically identical population and that of the genetically diverse population.

36. The only reasonable conclusion is (d). Statement

a. is not justified because the heritability value applies not to absolute height, nor to an individual, but to the variance in height among Southwestern undergraduates. Statement

b. is not justified because heritability has been determined only for Southwestern University students; students at other universities, with different ethnic backgrounds and from different regions of the country, may have different heritabilities of height. Statement

c. is, again, not justified, because heritability refers to the variance of height rather than to absolute height. Statement

e. is not justified because heritability has been determined for the range of variation in nongenetic factors experienced by the population under study; environmental variation outside this range (such as severe malnutrition) may have profound effects on height.

37. The regression coefficient and the narrow-sense heritability are each 0.8.

42.

a. Selection differential = 6 cm; response to selection = 3.6 cm.

b. The average wing length of the progeny should be 7.6 cm.

47. Head width will decrease. These two traits have high negative genetic correlation; therefore, selection for one trait will affect the other trait inversely.

Chapter 25

16. $f(T^ET^E) = 0.685$; $f(T^ET^F) = 0.286$; $f(T^FT^F) = 0.029$; $f(T^E) = 0.828$; $f(T^F) = 0.172$

17. $f(S^1S^1) = 0.023$; $f(S^1S^2) = 0.216$; $f(S^2S^2) = 0.761$; $f(S^1) = 0.13$; $f(S^2) = 0.87$

18.

$f(X^O) = 128/(128 + 370) = 0.26$

$f(X^+) = 370/498 = 0.74$

23.

a. $f(A) = 0.62$

$f(G) = 0.38$

b. Expected genotypic frequencies:

$f(AA) = 0.384$

$f(AG) = 0.471$

$f(GG) = 0.144$

c.

Genotype	Observed	Expected	$O - E$	$(O - E)^2$	$(O - E)^2/E$
AA	42	33	9	81	2.45
AG	24	41	17	289	7.05
GG	21	13	8	64	4.92

$$\chi = \sum (O - E)^2/E = 14.42$$

The degrees of freedom is 3 − 2 = 1.

The p value is much less than 0.05; therefore, we reject the hypothesis that these genotypic frequencies may be expected under Hardy–Weinberg equilibrium.

26. The frequency of heterozygous carriers = $2pq = 2(0.983)(0.017) = 0.033$; approximately 1 in 30 are carriers.

29.

a. 0.64 for M, 0.32 for MN, and 0.04 for N.

b. $f(L^M\ L^M) = 0.648$

$f(L^M\ L^N) = 0.304$

$f(L^N\ L^N) = 0.048$

31. The frequency at equilibrium is 0.98.

35.

a. On the island before the migration, $q^2 = 0.4$; $q = 0.63$.

On the mainland, $q^2 = 0.81$; $q = 0.9$.

b. After the migration, $q_{new} = 0.68$.

37. The small population sizes and founder effects will cause strong genetic drift. The geneticists will find considerable variation between populations in allelic frequencies. Within populations, the same factors, coupled with inbreeding, will cause loss of genetic variation and a high degree of homozygosity.

39.

a. 0.032;

b. 0.145.

40. At equilibrium, the frequency of *AR* will be 0.58, and the frequency of *ST* will be 0.42.

43.

a. 0.0071;

b. 5×10^{-5}.

Chapter 26

21. The first illustration shows evolutionary differences accumulating gradually over time in each lineage after cladogenesis; cladogenesis occurs before diversification, and gradual diversification of the lineages occurs after cladogenesis. This pattern of evolution has been called phyletic gradualism. The second illustration shows diversification of body shape and wing pattern occurring relatively rapidly and is associated with cladogenesis. This pattern suggests that cladogenesis is responsible for bringing about evolutionary divergence. This pattern has been called punctuated equilibrium.

25. Ecological (different host plants) and temporal (different times of mating).

31.

a. The living eagle species most closely related to Haast's eagle are one group of the little eagle and the booted eagle, and they are equally related to Haast's eagle. Note that another group of little eagles is slightly more distantly related.

b. The most distantly related species is the changeable hawk eagle.

c. The golden eagle and Haast's eagle share a more recent common ancestor than does the chestnut-bellied hawk eagle and Haast's eagle. Thus, the golden eagle and Haast's eagle are more closely related, in spite of the fact that the chestnut-bellied hawk eagle is next to Haast's eagle in the phylogenetic tree.

35. Introns. Higher rates of substitution are typically observed in those gene regions that have the least function because natural selection limits variation in functional parts of genes. Although the $3'$ untranslated region of a gene does not encode amino acids, it does contain sequences that play a role in mRNA stability and translation. Within an intron, only sequences at the $5'$ end, $3'$ end, and branch point function in splicing.

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TABLE 3.1 Summary of important genetic terms

Term	Definition
Gene	An inherited factor (encoded in the DNA) that helps determine a characteristic
Allele	One of two or more alternative forms of a gene
Locus	A specific place on a chromosome occupied by an allele
Genotype	A set of alleles possessed by an individual organism
Homozygote	An individual organism possessing two of the same alleles at a locus
Heterozygote	An individual organism possessing two different alleles at a locus
Characteristic or character	An attribute or feature possessed by an organism
Phenotype or trait	The appearance or manifestation of a characteristic

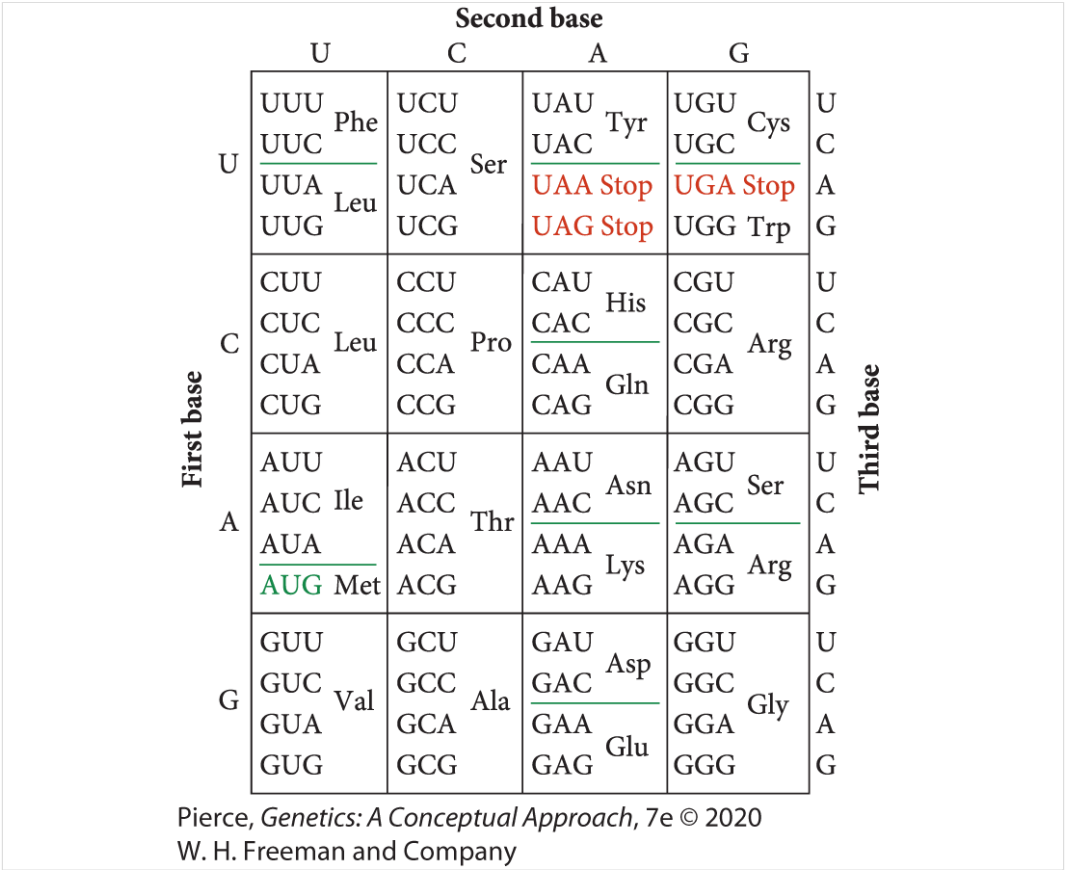

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FIGURE 15.10 The Genetic Code

Description

The column headers and left row headers for each of the four columns and rows are as follows U, C, A, and G. The right row headers for each row are U C A G, U C A G, U C A G, and U C A G. The combined left row heading is first base, combined column heading is second base, and combined right row heading is third base. The entries are depicted as follows:

Row 1 column 1: U U U and U U C (codes for phenylalanine); U U A and U U G (codes for leucine)

Row 1 column 2: U C U, U C C, U C A, U C G (codes for serine)

Row 1 column 3: U A U and U A C (codes for tyrosine); U A A and U A G (highlighted in red and represent stop codon)

Row 1 column 4: U G U and U G C (codes for cysteine); U G A (highlighted in red and represents stop codon); U G G (codes for tryptophan)

Row 2 column 1: C U U, C U C, C U A , C U G (codes for leucine)

Row 2 column 2: C C U, C C C, C C A, C C G (codes for proline)

Row 2 column 3: C A U and C A C (codes for histidine); C A A and C A G (codes for glutamine)

Row 2 column 4: C G U, C G C, C G A, C G G (codes for arginine)

Row 3 column 1: A U U, A U C, and A U A (codes for isoleusine); A U G (codes for methionine)

Row 3 column 2: A C U, A C C, A C A, A C G (codes for threonine)

Row 3 column 3: A A U and A A C (codes for asparagines); A A A and A A G (codes for lysine)

Row 3 column 4: A G U and A G C (codes for serine), A G A and A G G (codes for arginine)

Row 4 column 1: G U U, G U C, G U A, G U G (codes for valine)

Row 4 column 2: G C U, G C C, G C A, G C G (codes for alanine)

Row 4 column 3: G A U and G A C (codes for aspartic acid); G A A and G A G (codes for glutamic acid)

Row 4 column 4: G G U, G G C, G G A, G G G (codes for glycine).

TABLE 25.1 Extensions of the Hardy–Weinberg law

Situation	Allelic Frequencies	Genotypic Frequencies
Three alleles	$f(A^1) = p$	$f(A^1A^1) = p^2$

	$f(A^2) = q$	$f(A^1A^2) = 2pq$
	$f(A^3) = r$	$f(A^2A^2) = q^2$
		$f(A^1A^3) = 2pr$
		$f(A^2A^3) = 2qr$
		$f(A^3A^3) = r^2$
X-linked alleles	$f(X^1) = p$	$f(X^1X^1 \text{ female}) = p^2$
	$f(X^2) = q$	$f(X^1X^2 \text{ female}) = 2pq$
		$f(X^2X^2 \text{ female}) = q^2$
		$f(X^1Y \text{ male}) = p$
		$f(X^2Y \text{ male}) = q$

For X-linked female genotypes, the frequencies are the proportions among all females; for X-linked male genotypes, the frequencies are the proportions among all males.

TABLE 3.7 Critical values of the χ^2 distribution

	P								
df	**0.995**	**0.975**	**0.9**	**0.5**	**0.1**	**0.05***	**0.025**	**0.01**	**0.005**
1	0.000	0.000	0.016	0.455	2.706	3.841	5.024	6.635	7.879
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210	10.597
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345	12.838

4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277	14.860
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086	16.750
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812	18.548
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475	20.278
8	1.344	2.180	3.490	7.344	13.362	15.507	17.535	20.090	21.955
9	1.735	2.700	4.168	8.343	14.684	16.919	19.023	21.666	23.589
10	2.156	3.247	4.865	9.342	15.987	18.307	20.483	23.209	25.188
11	2.603	3.816	5.578	10.341	17.275	19.675	21.920	24.725	26.757
12	3.074	4.404	6.304	11.340	18.549	21.026	23.337	26.217	28.300
13	3.565	5.009	7.042	12.340	19.812	22.362	24.736	27.688	29.819
14	4.075	5.629	7.790	13.339	21.064	23.685	26.119	29.141	31.319
15	4.601	6.262	8.547	14.339	22.307	24.996	27.488	30.578	32.801

Notes

Chapter 2

[1]This analogy is adapted from K. Nasmyth, *Annual Review of Genetics* 35:673–745, 2001.