Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000.

Section 16.2 Electron Transport and Oxidative Phosphorylation

Most of the free energy released during the oxidation of glucose to CO_2 is retained in the reduced coenzymes NADH and $FADH_2$ generated during glycolysis and the citric acid cycle. During respiration, electrons are released from NADH and $FADH_2$ and eventually are transferred to O_2, forming H_2O according to the following overall reactions:

$$NADH + H^+ + \tfrac{1}{2}O_2 \longrightarrow NAD^+ + H_2O$$
$$FADH_2 + \tfrac{1}{2}O_2 \longrightarrow FAD + H_2O$$

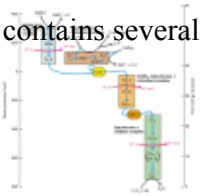
The $\Delta G^{\circ\prime}$ values for these strongly exergonic reactions are −52.6 kcal/mol (NADH) and −43.4 kcal/mol ($FADH_2$). Recall that the conversion of one glucose molecule to CO_2 via the glycolytic pathway and citric acid cycle yields 10 NADH and 2 $FADH_2$ molecules (see Table 16-1). Oxidation of these reduced coenzymes has a total $\Delta G^{\circ\prime}$ of −613 kcal/mol [10(−52.6) + 2(−43.4)]. Thus, of the potential free energy present in the chemical bonds of glucose (−680 kcal/mol), about 90 percent is conserved in the reduced coenzymes.

The free energy released during oxidation of a single NADH or $FADH_2$ molecule by O_2 is sufficient to drive the synthesis of several molecules of ATP from ADP and P_i, a reaction with a $\Delta G^{\circ\prime}$ of +7.3 kcal/mol. The mitochondrion maximizes the production of ATP by transferring electrons from NADH and $FADH_2$ through a series of electron carriers all but one of which are integral components of the inner membrane. This step-by-step transfer of electrons via the *electron transport chain* (also known as the *respiratory chain*) allows the free energy in NADH and $FADH_2$ to be released in small increments. At several sites during electron transport from NADH to O_2, protons from the mitochondrial matrix are transported uphill across the inner mitochondrial membrane and a proton concentration gradient forms across it (Figure 16-17). Because the outer membrane is freely permeable to protons, the pH of the mitochondrial matrix is higher (i.e., the proton concentration is lower) than that of the cytosol and intermembrane space. An electric potential across the inner membrane also results from the uphill pumping of positively charged protons outward from the matrix, which becomes negative with respect to the intermembrane space. Thus free energy released during the oxidation of NADH or $FADH_2$ is stored both as an electric potential and a proton concentration gradient — collectively, the proton-motive force — across the inner membrane. The movement of protons back across the inner membrane, driven by this force, is coupled to the synthesis of ATP from ADP and P_i by the F_0F_1 complex (see Figure 16-9).

Figure 16-17

Stepwise flow of electrons through the electron transport chain from NADH, succinate, and $FADH_2$ to O_2 (blue arrows). Each of the four large multiprotein complexes in the chain is located in the inner mitochondrial membrane and

contains several specific (more...)



The synthesis of ATP from ADP and P_i, driven by the transfer of electrons from NADH or $FADH_2$ to O_2, is the major source of ATP in aerobic nonphotosynthetic cells. Much evidence shows that in mitochondria and bacteria this process, called oxidative phosphorylation, depends on generation of an electrochemical proton gradient (i.e., proton-motive force) across the inner membrane, with electron transport, proton pumping, and ATP formation occurring simultaneously. In the laboratory, for instance, addition of O_2 and an oxidizable substrate such as pyruvate or succinate to isolated intact mitochondria results in a net synthesis of ATP if the inner mitochondrial membrane is intact. In the presence of minute amounts of detergents that make the membrane leaky, the oxidation of these metabolites by O_2 still occurs, but no ATP is made. Under these conditions, no transmembrane proton concentration gradient or membrane electric potential can be maintained.

In this section we first discuss the magnitude of the proton-motive force, then the components of the electron transport chain and the translocation of protons across the inner membrane. Next we describe the structure of the F_0F_1 complex and how it uses the proton-motive force to synthesize ATP. In the last section, we consider how mitochondrial oxidation of NADH and $FADH_2$ is controlled to meet the cell's need for ATP.

The Proton-Motive Force in Mitochondria Is Due Largely to a Voltage Gradient across the Inner Membrane

As we've seen, the proton-motive force (pmf) is the sum of a transmembrane proton concentration (pH) gradient and electric potential, or voltage gradient.* The relative contribution of the two components to the total pmf depends on the permeability of the membrane to ions other than H^+. A significant voltage gradient can develop only if the membrane is poorly permeable to other cations and to anions, as is the inner mitochondrial membrane. In this case, the developing voltage gradient (i.e., excess H^+ ions on the intermembrane face and excess anions on the cytosolic face) soon prevents further proton movement, so only a small pH gradient is generated. In contrast, a significant pH gradient can develop only if the membrane is also permeable to a major anion (e.g., Cl^-), or if the protons are exchanged for another cation (e.g., K^+). In either case, proton movement does not lead to a voltage gradient across the membrane because there is always an equal concentration of positive and negative ions on each side of the membrane. This is the situation in the chloroplast thylakoid membrane during photosynthesis, as we discuss later. Compared with chloroplasts, then, a greater portion of the pmf in mitochondria is due to the membrane electric potential, and the actual pH gradient is smaller.

Since a difference of one pH unit represents a tenfold difference in H^+ concentration, a pH

gradient of one unit across a membrane is equivalent to an electric potential of 59 mV (at 20 °C). Thus we can define the proton-motive force, pmf, as

$$\text{pmf} = \Psi - \left(\frac{RT}{\mathcal{F}}\right) \times \Delta\text{pH} = \Psi - 59\Delta\text{pH}$$

where R is the gas constant of 1.987 cal/(degree·mol), T is the temperature (in degrees Kelvin), $\mathcal{F}$ is the Faraday constant [23,062 cal/ (V·mol)], and Ψ is the transmembrane electric potential; Ψ and pmf are measured in millivolts. Measurements on respiring mitochondria have shown that the electric potential (Ψ) across the inner membrane is ≈160 mV (negative inside matrix) and that ΔpH is ≈1.0 (equivalent to ≈60 mV). Thus the total pmf is ≈220 mV, with the transmembrane electric potential responsible for about 73 percent.

Because mitochondria are much too small to be impaled with electrodes, the electric potential and pH gradient across the inner mitochondrial membrane cannot be determined by direct measurement. By trapping fluorescent pH-sensitive dyes inside vesicles formed from the inner mitochondrial membrane, researchers can measure the inside pH during oxidative phosphorylation. The electric potential can be determined by adding radioactive K^+ ($^{42}K^+$) ions and a trace amount of valinomycin (K^+ **ionophore**) to a suspension of respiring mitochondria. Although the inner membrane is normally impermeable to K^+, valinomycin is a lipid-soluble peptide that can selectively bind K^+ in its hydrophilic interior and carry it across otherwise impermeable membranes. $^{42}K^+$ will equilibrate across the membrane in accordance with the electric potential; the more negative the matrix side of the inner membrane, the more $^{42}K^+$ will accumulate. Thus, the concentration of radioactive K^+ ions in the matrix and cytosol is measured after equilibrium is reached. From the measured $^{42}K^+_{\text{matrix}}$:$^{42}K^+_{\text{cytosol}}$ ratio, the electric potential E (in mV) across the inner membrane can be calculated using the Nernst equation (Equation 15-5). When trace amounts of valinomycin and radioactive potassium ($^{42}K^+$) ions are added to a suspension of respiring mitochondria, oxidative phosphorylation proceeds and is largely unaffected. The $^{42}K^+$ ions accumulate inside the mitochondria in a K_{matrix}:K_{cytosol} ratio of about 500. By substituting this value in the Nernst equation,

$$E = -59 \log \frac{[K_{in}]}{[K_{out}]} = -59 \log 500 = -160 \text{ mV}$$

we can see that the electric potential across the inner membrane is ≈160 mV, with the inside negative.

Electron Transport in Mitochondria Is Coupled to Proton Translocation

As noted earlier, the stepwise movement of electrons from NADH and $FADH_2$ to O_2 via a series of electron carriers is coupled to translocation of protons from the mitochondrial matrix to the intermembrane space. This proton movement generates the proton-motive force that directly powers ATP synthesis. That electron transport from NADH (or $FADH_2$) to O_2 is coupled to proton transport across the membrane is demonstrated by the experiment depicted in Figure 16-18. As soon as O_2 is added to a suspension of mitochondria, the medium outside the mitochondria becomes acidic. During electron transport from NADH to O_2, protons translocate

from the matrix to the intermembrane space; since the outer membrane is freely permeable to protons, the pH of the outside medium is lowered briefly. The measured change in pH indicates that 10 protons are transported out of the matrix for every electron pair transferred from NADH to O_2.

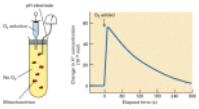


Figure 16-18

Experimental demonstration that electron transport from NADH or $FADH_2$ to O_2 is coupled to proton transport across the membrane. If a source of electrons for respiration, such as NADH, is added to a suspension of mitochondria depleted of O_2, no NADH is oxidized. (more...)

When this experiment is repeated with succinate rather than NADH as the reduced substrate, the medium outside the mitochondria again becomes acidic, but less so. Recall that oxidation of succinate to fumarate in the citric acid cycle generates $FADH_2$(see Figure 16-12). Because $FADH_2$ transfers electrons to the electron transport chain at a later point than NADH does, electron transport from $FADH_2$ (or succinate) results in translocation of fewer protons from the matrix, and thus a smaller change in pH (see Figure 16-17).

Electrons Flow from $FADH_2$ and NADH to O_2 via a Series of Multiprotein Complexes

We now examine more closely the energetically favored movement of electrons from the coenzymes NADH and $FADH_2$ to the final electron acceptor, O_2. In respiring mitochondria, each NADH molecule releases two electrons to the electron transport chain; these electrons ultimately reduce one oxygen atom (half of an O_2 molecule), forming one molecule of water:

$$NADH \longrightarrow NAD^+ + H^+ + 2e^-$$

$$2e^- + 2H^+ + \tfrac{1}{2}O_2 \longrightarrow H_2O$$

As electrons move from NADH to O_2, their potential declines by 1.14 V, which corresponds to 26.2 kcal/mol of electrons transferred, or ≈53 kcal/mol for a pair of electrons. Much of this energy is conserved at three stages of electron transport by the movement of protons across the inner mitochondrial membrane from the matrix to the intermembrane space (see Figure 16-17).

Most mitochondrial electron carriers comprise prosthetic groups, such as flavins, heme, iron-sulfur clusters, and copper, bound to four multiprotein complexes, each of which spans the inner mitochondrial membrane (Figure 16-19). Table 16-2 lists the prosthetic groups in each multiprotein complex. Before considering the function of each complex, we take a detailed look at some of the individual carriers in the electron transport chain.

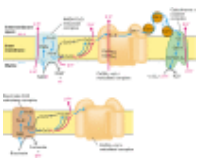


Figure 16-19

The pathway of electron transport (blue arrows) and proton transport (red arrows) in the inner mitochondrial membrane.

Bound to each of the electron transport complexes are several prosthetic groups, which carry electrons through the complex. *(Top)* A (more...)

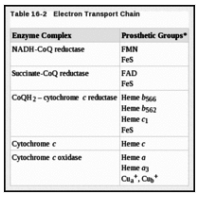


Table 16-2

Electron Transport Chain.

Enzyme Complex	Prosthetic Groups*
NADH-CoQ reductase	FMN FeS
Succinate-CoQ reductase	FAD FeS
$CoQH_2$ – cytochrome *c* reductase	Heme b_{566} Heme b_{562} Heme c_1 FeS
Cytochrome *c*	Heme *c*
Cytochrome *c* oxidase	Heme *a* Heme a_3 Cu_A^{2+}, Cu_B^{2+}

Iron-sulfur clusters, Fe_2S_2 and Fe_4S_4, are nonheme prosthetic groups consisting of Fe atoms bonded both to inorganic S atoms and to four S atoms on cysteine residues on the protein (Figure 16-20). Some Fe atoms in the cluster bear a +2 charge while others have a +3 charge. However, the net charge of each Fe atom is actually between +2 and +3, because electrons in the outermost orbits are dispersed among the Fe atoms and move rapidly from one atom to another. Iron-sulfur clusters accept and release electrons one at a time; the additional electron is also dispersed over all the Fe atoms in the cluster.

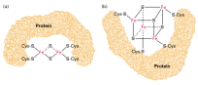


Figure 16-20

Three-dimensional structures of some iron-sulfur clusters in electron-transporting proteins: (a) a dimeric (Fe_2S_2) cluster; (b) a tetrameric (Fe_4S_4) cluster. In both types of clusters, each Fe atom is bonded to four S atoms: some S atoms are molecular (more...)

The cytochromes are proteins covalently linked to a heme molecule, an iron-containing prosthetic group similar to that in hemoglobin or myoglobin. Electron transport occurs by oxidation and reduction of the Fe atom in the center of the heme:

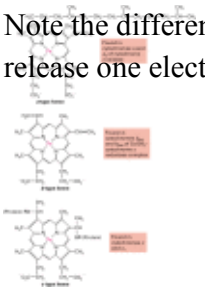
$$Fe^{3+}_{ox} + e^- \rightleftharpoons Fe^{2+}_{red}$$

In the electron transport chain, electrons move through the cytochromes in the following order: b_{566}, b_{562}, c_1, *c, a,* and a_3 (see Figure 16-19). The various cytochromes have slightly different heme groups and axial ligands, which generate different environments for the Fe ion (Figure 16-21). Therefore, each cytochrome has a different reduction potential, or tendency to accept an electron — an important property dictating the unidirectional electron flow along the chain. Because the heme ring in cytochromes consists of alternating double- and single-bonded atoms, a large number of resonance forms exist, and the extra electron is delocalized to the heme carbon and nitrogen atoms as well as to the Fe ion.

Figure 16-21

Heme prosthetic groups of respiratory-chain cytochromes in mitochondria.

Note the differences in substituents on the porphyrin rings. Hemes accept and release one electron at a time.



Coenzyme Q (CoQ), also called *ubiquinone,* is the only electron carrier that is not a protein-bound prosthetic group. It is a carrier of hydrogen atoms (protons plus electrons). The oxidized quinone form of CoQ can accept a single electron to form a semiquinone, and then a second electron and two protons to form the fully reduced form, dihydroubiquinone (Figure 16-22). Both CoQ and the reduced form $CoQH_2$ are soluble in phospholipids and diffuse freely in the inner mitochondrial membrane. CoQ accepts electrons released from the NADH-CoQ reductase complex and the succinate-CoQ reductase complex (see Figure 16-17).

Figure 16-22

The structure of coenzyme Q (CoQ), also called *ubiquinone,* illustrating its ability to carry two protons and two electrons. Found in bacterial and mitochondrial membranes, CoQ is the only carrier in the electron transport chain that is not tightly bound (more...)

NADH-CoQ Reductase Complex

Electrons are carried from NADH to CoQ by the NADH-CoQ reductase complex. NAD^+ is exclusively a two-electron carrier: it accepts or releases a pair of electrons at a time. In the NADH-CoQ reductase complex, electrons first flow from NADH to FMN (flavin mononucleotide), a cofactor related to FAD, and then to an iron-sulfur protein (see Figure 16-19). FMN, like FAD, can accept two electrons, but does so one electron at a time (see Figure 16-8).

The overall reaction catalyzed by this complex is

$$\underset{\text{(Reduced)}}{NADH} + \underset{\text{(Oxidized)}}{CoQ} + 2H^+ \longrightarrow \underset{\text{(Oxidized)}}{NAD^+} + H^+ + \underset{\text{(Reduced)}}{CoQH_2}$$

Each transported electron undergoes a drop in potential of ≈360 mV, equivalent to a $\Delta G^{\circ\prime}$ of −16.6 kcal/mol for the two electrons transported (see Figure 16-17). Much of this released energy is used to transport four protons across the inner membrane per molecule of NADH oxidized by the NADH-CoQ reductase complex, but how this protein transport occurs is not know.

Succinate-CoQ Reductase Complex

As mentioned earlier, succinate dehydrogenase, the enzyme that oxidizes a molecule of succinate to fumarate in the citric acid cycle, is localized to the inner mitochondrial membrane (see Figure 16-12, step 7). Actually, this enzyme is an integral component of the succinate-CoQ reductase complex. The two electrons released in conversion of succinate to fumarate are transferred first to FAD, then to an iron-sulfur carrier, and finally to CoQ, forming the reduced $CoQH_2$(see Figure 16-19). The overall reaction catalyzed by this complex is

$$\underset{\text{(Reduced)}}{\text{Succinate}} + \underset{\text{(Oxidized)}}{\text{CoQ}} \longrightarrow \underset{\text{(Oxidized)}}{\text{fumarate}} + \underset{\text{(Reduced)}}{\text{CoQH}_2}$$

Although the $\Delta G^{\circ\prime}$ for this reaction is negative, the released energy is insufficient for proton pumping. Thus no protons are translocated across the membrane by the succinate-CoQ reductase complex, and no proton-motive force is generated in this part of the electron transport chain.

$CoQH_2$—Cytochrome *c* Reductase Complex

Reduced $CoQH_2$, generated by either oxidation of NADH or succinate, donates two electrons to the $CoQH_2$ – cytochrome *c* reductase complex, regenerating oxidized CoQ. Within this complex the released electrons are transferred to an iron-sulfur protein and to two *b*-type cytochromes, then to cytochrome c_1. Finally, the two electrons are transferred to two molecules of the oxidized form of cytochrome *c* (a water-soluble intermembrane-space protein), forming reduced cytochrome *c* (see Figure 16-19). For each pair of electrons transferred, the overall reaction catalyzed by the $CoQH_2$ – cytochrome *c* reductase complex is

$$\underset{\text{(Reduced)}}{CoQH_2} + 2\ \underset{\text{(Oxidized)}}{\text{Cyt } c^{3+}} \longrightarrow \underset{\text{(Oxidized)}}{CoQ} + 2\ H^+ + 2\ \underset{\text{(Reduced)}}{\text{Cyt } c^{2+}}$$

The $\Delta G^{\circ\prime}$ for this reaction is sufficiently negative that four protons are translocated from the mitochondrial matrix across the inner membrane for each pair of electrons transferred; this involves the proton-motive Q cycle discussed later.

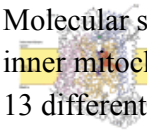
Cytochrome c Oxidase Complex

Cytochrome *c,* after being reduced by the $CoQH_2$ – cytochrome *c* reductase complex, transports electrons, one at a time, to the cytochrome *c* oxidase complex (Figure 16-23). Within this complex, electrons are transferred, again one at a time, first to a pair of copper ions (Cu_a^{2+}), then to cytochrome *a,* then to a complex of a second copper ion (Cu_b^{2+}), and cytochrome a_3, and finally to O_2, the ultimate electron acceptor, yielding H_2O. For each pair of electrons transferred, the overall reaction catalyzed by the cytochrome *c* oxidase complex is

$$\underset{\text{(Reduced)}}{2\ \text{Cyt } c^{2+}} + 2\ H^+ + \tfrac{1}{2}\ O_2 \longrightarrow \underset{\text{(Oxidized)}}{2\ \text{Cyt } c^{3+}} + H_2O$$

During transport of each pair of electrons through the cytochrome *c* oxidase complex, two protons are translocated across the membrane.

Figure 16-23



Molecular structure of the core of the cytochrome *c* oxidase complex in the inner mitochondrial membrane. Mitochondrial cytochrome *c* oxidases contain 13 different subunits, but the catalytic core of the enzyme consists of only 3 subunits: I (yellow) II (more...)

CoQ and Cytochrome *c* Shuttle Electrons from One Electron Transport Complex to Another

Each of the four electron transport complexes just described are laterally mobile in the inner mitochondrial membrane. The complexes are present in nonequal amounts: for each NADH-CoQ reductase complex, there are about three $CoQH_2$ – cytochrome *c* reductase complexes and seven cytochrome *c* oxidase complexes. Furthermore, there do not appear to be stable contacts between any two complexes; rather, electrons are transported from one complex to another only by diffusion of CoQ and cytochrome *c,* which act as electron shuttles (see Figure 16-19). Because CoQ is lipid-soluble, it can diffuse in the membrane, shuttling electrons picked up from the NADH-CoQ reductase and succinate-CoQ reductase complexes to the $CoQH_2$ – cytochrome *c* reductase complex. After oxidized cytochrome *c* picks up an electron from the $CoQH_2$ – cytochrome *c* reductase complex, the reduced carrier diffuses in the intermembrane space until it encounters a cytochrome *c* oxidase complex, to which it donates an electron. Mitochondrial electron flow, in summary, does not resemble an electric current through a wire, with each electron following the previous one. Rather, electrons are picked up by a carrier, one or two at a time, and then passed along to the next carrier in the pathway.

Reduction Potentials of Electron Carriers Favor Electron Flow from NADH to O_2

As we saw in Chapter 2, the reduction potential ***E*** for a partial reduction reaction

$$\text{oxidized molecule} + e^- \rightleftharpoons \text{reduced molecule}$$

is a measure of the equilibrium constant of that partial reaction. With the exception of the *b* cytochromes in the $CoQH_2$ – cytochrome *c* reductase complex, the standard reduction potential E'_0 of the carriers in the mitochondrial electron transport chain increases steadily from NADH to O_2.

For instance, for the partial reaction

$$NAD^+ + H^+ + 2\,e^- \rightleftharpoons NADH$$

the value of the standard reduction potential is −320 mV, which is equivalent to a $\Delta G°'$ of +14.8 kcal/mol for transfer of two electrons (see Table 2-7). Thus this partial reaction tends to proceed toward the left; that is, toward the oxidation of NADH to NAD^+.

By contrast, the standard reduction potential for the partial reaction

$$\text{Cyt } c_{ox}(Fe^{3+}) + e^- \rightleftharpoons \text{Cyt } c_{red}(Fe^{2+})$$

is +220 mV ($\Delta G^{\circ\prime} = -5.1$ kcal/mol) for transfer of one electron. Thus this partial reaction tends to proceed toward the right; that is, toward the reduction of cytochrome *c* (Fe^{3+}) to *c* (Fe^{2+}).

The final reaction in the electron transport chain, the reduction of O_2 to H_2O

$$2\,H^+ + \tfrac{1}{2}\,O_2 + 2\,e^- \longrightarrow H_2O$$

has a standard reduction potential of +816 mV ($\Delta G^{\circ\prime} = -37.8$ kcal/mol for transfer of two electrons), the most positive in the whole series, and thus also tends to proceed toward the right.

As illustrated in Figure 16-17, the steady increase in E'_0 values, and the corresponding decrease in ΔG values, of the carriers in the electron transport chain favors the flow of electrons from NADH and succinate to oxygen.

CoQ and Three Electron Transport Complexes Pump Protons out of the Mitochondrial Matrix

The experiment depicted in Figure 16-18 demonstrated that electron transport in intact mitochondria is coupled to proton export, leading to an increase in the H^+ concentration of the surrounding medium. The multiprotein electron transport complexes responsible for proton pumping have been identified by selectively extracting mitochondrial membranes with detergents, isolating each of the complexes in near purity, and then preparing artificial phospholipid vesicles (liposomes) containing each complex (see Figure 15-4). When an appropriate electron donor and electron acceptor are added to such liposomes, a change in pH of the medium will occur if the embedded complex transports protons.

For example, the cytochrome *c* oxidase complex can be incorporated into liposomes so that the binding site for cytochrome *c* is on the outside (Figure 16-24). Direct measurements of the pH change that occurs when reduced cytochrome *c* and O_2 are added indicate that two protons are transported out of the vesicles for every electron pair transported (or, equivalently, for every two molecules of cytochrome *c* oxidized). Similar studies indicate that the NADH-CoQ reductase complex and $CoQH_2$ – cytochrome *c* reductase complex each translocates four protons per pair of electrons transported.

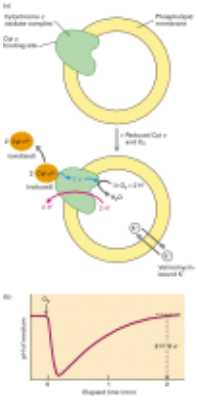


Figure 16-24

Experimental demonstration that oxidation of reduced cytochrome *c* (Cyt c^{2+}) by the cytochrome *c* oxidase complex is coupled to proton transport. (a) The oxidase complex is incorporated into liposomes with the binding site for cytochrome *c* positioned on (more...)

Current evidence thus suggests that a total of 10 protons are transported from the matrix space

across the inner mitochondrial membrane for every electron pair that is transferred from NADH to O_2. Since the succinate-CoQ reductase complex does not transport protons, only six protons are transported across the membrane for every electron pair that is transferred from succinate (or $FADH_2$) to O_2. (Note that the protons generated in the matrix by the oxidation of NADH to NAD^+ and $H^+$are consumed by the cytochrome *c* oxidase complex during the formation of H_2O, resulting in no net movement of protons across the membrane.) Relatively little is known about the mechanism of proton translocation by the NADH-CoQ reductase complex, but the mechanism of proton translocation by the two cytochrome complexes is well understood.

Proton-Motive Q Cycle

Four protons are translocated across the membrane per electron pair transported through the $CoQH_2$ – cytochrome *c* reductase complex. Coenzyme Q (CoQ) plays a key role in this translocation process, which is known as the *proton-motive Q cycle,* or *Q cycle.* During this process, CoQ cycles between its reduced and oxidized states by accepting and releasing two protons and two electrons together:

$$CoQ + 2\,H^+ + 2\,e^- \rightleftharpoons CoQH_2$$

Figure 16-25 depicts the cycle, which begins when a molecule of CoQ binds to a site, located near the matrix surface of the NADH-CoQ reductase complex (or succinate-CoQ reductase complex), and picks up two protons from the *matrix space* and two electrons. The reduced $CoQH_2$ diffuses randomly in the membrane, but eventually binds to a site on the *intermembrane side* of the $CoQH_2$ – cytochrome *c* reductase complex, where it releases two protons into the intermembrane space (step 1); these represent two of the four protons translocated from the matrix to the intermembrane space per pair of electrons transported. Simultaneously, one of the two electrons from $CoQH_2$ is transported, via an iron-sulfur protein and cytochrome c_1, directly to cytochrome *c* (steps 2a, 3, and 4). The other electron released from the $CoQH_2$, called a *cycling electron,* moves through cytochromes b_{566} and b_{562} to another CoQ binding site on the matrix surface, where it reduces a bound oxidized CoQ molecule (steps 2b, 5, and 6a). This reaction forms the partially reduced CoQ semiquinone anion, denoted by $CoQ^{-}\cdot$ (see Figure 16-22). When a second cycling electron, released from a second $CoQH_2$, is similarly transported through cytochromes b_{566} and b_{562}, it reduces the bound $CoQ^{-}\cdot$ in a reaction that uses two protons picked up from the matrix space to form $CoQH_2$ (step 6b). This $CoQH_2$ molecule then is released from the $CoQH_2$ –cytochrome *c* reductase complex and diffuses through the membrane to the CoQ-binding site on the intermembrane surface of the same complex, where it releases its two protons into the intermembrane space (step 7), as well as one electron directly to cytochromes c_1 and *c,* and one electron to recycle through cytochromes b_{566} and b_{562}.

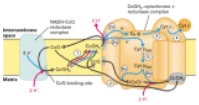


Figure 16-25

The proton-motive Q cycle. Binding sites for CoQ on the protein complexes are indicated by gray shading. One electron from $CoQH_2$ travels to cytochrome *c* via steps 2a, 3,

and 4; the other cycles through the *b* cytochromes. The net result is that four protons (more...)

To calculate the number of protons transported from the matrix space to the intermembrane space per pair of electrons transported through the $CoQH_2$ – cytochrome *c* reductase complex, imagine 100 molecules of $CoQH_2$ interacting with $CoQH_2$ – cytochrome *c* reductase complexes. In the first passage through the cycle, 200 protons will be transported across the membrane into the intermembrane space and 200 electrons will be released. Of the released electrons, 100 will be transported directly to cytochrome c_1 and 100 will cycle through the *b* cytochromes, generating 50 new molecules of reduced $CoQH_2$. In the second cycle, these 50 molecules of $CoQH_2$ will transport 100 protons into the intermembrane space and generate 50 cycling electrons, which will in turn generate 25 new molecules of reduced $CoQH_2$. Continuing this process ad infinitum, the number of protons transported as a result of oxidation of the original 100 molecules of $CoQH_2$ by 200 electrons will be

$$200 + 100 + 50 + 25 + 12.5 + 6.25 + 3.125 + \cdots = 400$$

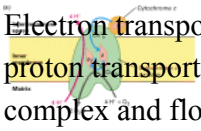
Since we started with 100 molecules of $CoQH_2$, this exercise demonstrates that for every *pair of electrons* transported from $CoQH_2$ through the $CoQH_2$ – cytochrome *c* reductase complex to cytochrome *c, four protons* are translocated across the membrane — two released by the initial $CoQH_2$, and two by electrons cycling through the *b* cytochromes. The ability of electrons to cycle through the *b* cytochromes thus doubles the number of protons translocated.

Coupling of H^+ Pumping and O_2 Reduction by Cytochrome *c* Oxidase

After cytochrome *c* is reduced by the $CoQH_2$ – cytochrome *c* reductase complex, it is reoxidized by the cytochrome *c* oxidase complex. The oxidation of four cytochrome *c* molecules is coupled to the reduction of one molecule of O_2, forming two molecules of water. As we saw earlier, cytochrome *c* oxidase contains three copper ions and two heme groups; the oxygen-reduction center consists of one molecule of heme a_3 and one copper ion bound to subunit I of the cytochrome *c* oxidase complex (see Figure 16-23).

The flow of electrons through cytochrome *c* oxidase is shown in Figure 16-26a. Four molecules of reduced cytochrome *c* bind, one at a time, to a site on subunit II of the oxidase. An electron is transferred from the heme of each cytochrome *c,* first to Cu_a^{2+} bound to subunit II, then to the heme *a* bound to subunit I, and finally to the Cu_b^{2+} and heme a_3 in the oxygen-reduction center. The cyclic oxidation and reduction of the iron and copper in the reduction center, together with the uptake of four protons from the matrix space, is coupled to the transfer of the four electrons to oxygen and the formation of water (Figure 16-26b). Proposed intermediates in oxygen reduction include the peroxide anion (O_2^{2-}) and probably the hydroxyl radical (OH•). These intermediates would be harmful if they escaped from the reaction center, but they do so only rarely.

Figure 16-26



Electron transport through the cytochrome *c* oxidase complex and coupled proton transport. (a) Arrangement of electron carriers within the oxidase complex and flow of electrons from reduced cytochrome *c* to O_2. All the heme groups are shown in red. From (more...)

For every four electrons transferred from reduced cytochrome *c* through the cytochrome *c* oxidase complex (i.e., for every molecule of O_2 reduced to two H_2O molecules), four additional protons are translocated from the matrix space to the intermembrane space (two protons per electron pair). The four protons move during steps 2, 5, and 6 of the cycle depicted in Figure 16-26b, but the mechanism by which these protons are translocated is not known.

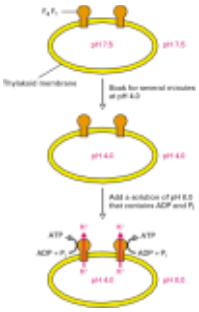
Experiments with Membrane Vesicles Support the Chemiosmotic Mechanism of ATP Formation

The hypothesis that a proton-motive force is the immediate source of energy for ATP synthesis, introduced in 1961 by Peter Mitchell, was initially opposed by virtually all researchers working in oxidative phosphorylation and photosynthesis. They favored a mechanism similar to the well-elucidated substrate-level phosphorylation in glycolysis, in which oxidation of a substrate molecule is directly coupled to ATP synthesis (see Figure 16-3, steps 6 and 9). By analogy, electron transport through the membranes of chloroplasts or mitochondria was believed to generate an intermediate containing a high-energy chemical bond (e.g., a phosphate linked to an enzyme by an ester bond), which was then used to convert P_i and ADP to ATP. Despite intense efforts by a large number of investigators, however, no such intermediate could ever be identified.

Definitive evidence supporting the role of the proton-motive force in ATP synthesis awaited the development of techniques to purify and reconstitute organelle membranes and membrane proteins. One experiment that led to general acceptance of Mitchell's chemiosmotic mechanism is outlined in Figure 16-27. Chloroplast thylakoid vesicles containing F_0F_1 particles were equilibrated in the dark with a buffered solution at pH 4.0. When the pH in the thylakoid lumen became 4.0, the vesicles were rapidly mixed with a solution at pH 8.0 containing ADP and P_i. A burst of ATP synthesis accompanied the transmembrane movement of protons driven by the 10,000-fold concentration gradient (10^{-4} M versus 10^{-8} M). In reciprocal experiments using "inside-out" preparations of submitochondrial vesicles, an artificially generated membrane electric potential also resulted in ATP synthesis. These findings left no doubt that the F_0F_1 complex is the ATP-generating enzyme and that ATP generation is dependent on proton movement down its electrochemical gradient.

Figure 16-27

Experimental demonstration that ATP synthesis from ADP and P_i in chloroplast thylakoid membranes results from an artificially imposed pH gradient.



Bacterial Plasma-Membrane Proteins Catalyze Electron Transport and Coupled ATP Synthesis

Although bacteria lack mitochondria, aerobic bacteria nonetheless carry out oxidative phosphorylation by the same processes that occur in eukaryotic mitochondria. Enzymes that catalyze the reactions of both the glycolytic pathway and the citric acid cycle are present in the cytosol of bacteria; enzymes that oxidize NADH to NAD^+ and transfer the electrons to the ultimate acceptor O_2 are localized to the bacterial plasma membrane.

The movement of electrons through these membrane carriers is coupled to the pumping of protons out of the cell (see Figure 16-2). The movement of protons back into the cell, down their concentration gradient, is coupled to the synthesis of ATP. Bacterial F_0F_1 complexes are essentially identical in structure and function with the mitochondrial F_0F_1 complex, which we examine in some detail below. The proton-motive force across the bacterial plasma membrane is also used to power the uptake of nutrients such as sugars and the rotation of bacterial flagella (see Figure 16-1).

ATP Synthase Comprises a Proton Channel (F_0) and ATPase (F_1)

The F_0F_1 complex, or ATP synthase, has two principal components, F_0 and F_1, both of which are oligomeric proteins (Figure 16-28). F_0 is located within the membrane and contains a transmembrane channel through which protons flow toward F_1, most of which extends into the mitochondrial matrix (or cytosol in bacteria).

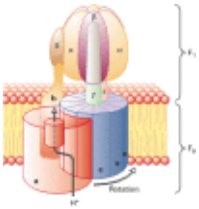


Figure 16-28

Model of the structure of ATP synthase (the F_0F_1 ATPase complex) in the bacterial plasma membrane. The F_0 portion is built of three integral membrane proteins: *a*, *b*, and 9 – 12 copies of *c* arranged in a ring in the plane of the (more...)

The F_0 component contains three types of subunits, **a**, **b**, and **c**; in bacteria, the subunit composition is $\mathbf{a}_1\mathbf{b}_2\mathbf{c}_{9–12}$. The **c** subunits form a donut-shaped ring in the plane of the membrane. Each **a** subunit is thought to span the membrane eight times, each **b** once, and each **c** twice. In mitochondria, each F_0 complex also contains, depending on the species, two to five additional peptides of unknown function. When F_0 is experimentally incorporated into

liposomes, the permeability of the vesicles to H^+ is greatly stimulated, indicating that it indeed forms a proton channel. Each copy of subunit **c** contains two membrane-spanning α helices; an aspartate residue in one of these helices is thought to participate in proton movement, since chemical modification of this aspartate by dicyclohexylcarbodiimide or its mutation specifically blocks H^+ translocation. The proton channel lies at the interface of the **a** and **c** subunits (see Figure 16-28).

The F_1 portion is a water-soluble complex of five distinct polypeptides with the composition $\alpha_3\beta_3\gamma\delta\epsilon$. The α and β subunits associate in alternating order to form a hexamer, αβαβαβ or $(\alpha\beta)_3$. This hexamer rests atop the single long γ subunit, whose lower part is a coiled coil that fits into the **c**-subunit ring of F_0. The ϵ subunit is attached to γ and probably also contacts the **c** subunits of F_0. The δ subunit of the F_1 complex contacts the **b** subunit of the F_0 complex; together these subunits form a rigid "stator" that prevents the $(\alpha\beta)_3$ hexamer from rotating while it rests on the γ subunit (see Figure 16-28).

F_1 forms the knobs that protrude from the matrix side of the inner membrane. When physically separated from the membrane by mechanical agitation, F_1 is capable only of catalyzing ATP hydrolysis. Hence, it has been called the F_1 ATPase, but its natural function is synthesis of ATP. Submitochondrial vesicles from which F_1 is removed cannot catalyze ATP synthesis; when F_1 particles reassociate with these vesicles, they once again become fully active in ATP synthesis (Figure 16-29).

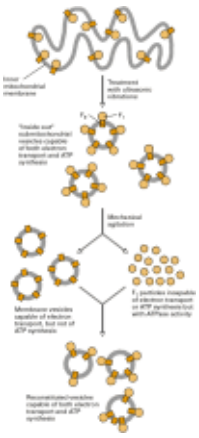


Figure 16-29

Experimental demonstration that mitochondrial F_1 particles are required for ATP synthesis, but not for electron transport. Exposure of the inner mitochondrial membrane to ultrasonic vibration disrupts the membrane; the fragments reseal with the F_1 particles (more...)

Each of the three β subunits in the complete F_0F_1 complex can bind ATP, ADP, and P_i, and catalyze ATP synthesis. However, the coupling between proton flow and ATP synthesis must be indirect, since the nucleotide-binding sites on the β subunits of F_1, where ATP synthesis occurs, are 9 – 10 nm from the surface of the mitochondrial membrane. In the next section, we examine this coupling mechanism.

The F_0F_1 Complex Harnesses the Proton-Motive Force to Power ATP Synthesis

The most widely accepted model for ATP synthesis by the F_0F_1 complex — the *binding-change mechanism* — posits that the energy released by the downhill movement of protons through F_0

directly powers rotation of the γ subunit and attached ϵ subunit. Most likely the γ and ϵ subunits rotate together with the ring of **c** subunits, relative to the fixed **a** subunit, but it is possible that the γ subunit rotates in the center of a fixed ring of **c** subunits. In either case, the γ subunit acts as cam, a rotating shaft within F_1 whose movement causes cyclical changes in the conformations of the β subunits. As schematically depicted in Figure 16-30, rotation of the γ subunit relative to the fixed $(\alpha\beta)_3$ hexamer causes the nucleotide-binding site of each β subunit to cycle through three conformational states in the following order:

1. An O state that binds ATP very poorly and ADP and P_i weakly
2. An L state that binds ADP and P_i more strongly
3. A T state that binds ADP and P_i so tightly that they spontaneously form ATP and that binds ATP very strongly

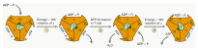


Figure 16-30

The binding-change mechanism of ATP synthesis from ADP and P_i by the F_0F_1 complex. This view is looking up at F_1 from the membrane surface (see Figure 16-28). The three β subunits alternate between three conformational states that differ in their (more...)

A final rotation of γ returns the β subunit to the O state, thereby releasing ATP and beginning the cycle again. (ATP or ADP also bind to regulatory or allosteric sites on the three α subunits; this binding modifies the rate of ATP synthesis according to the level of ATP and ADP in the matrix, but is not directly involved in synthesis of ATP from ADP and P_i.)

When first proposed, the binding-change mechanism was not generally accepted, but much evidence has accumulated to support it. First, biochemical studies showed that one of the three β subunits on isolated F_1 particles can tightly bind ADP and P_i and then form ATP, which remains tightly bound. The measured $\Delta G^{\circ\prime}$ for this reaction is near zero, indicating that once ADP and P_i are bound to what is now called the T state of a β subunit, they spontaneously form ATP. Importantly, dissociation of the bound ATP from the β subunit on isolated F_1 particles occurs extremely slowly. This finding suggested that dissociation of ATP would have to be powered by a conformational change in the β subunit, which, in turn, would be caused by proton movement.

Later x-ray crystallographic analysis of the $(\alpha\beta)_3$ hexamer yielded a striking conclusion: although the three β subunits are identical in sequence and overall structure, the ADP/ATP-binding sites have different conformations in each subunit. The most reasonable conclusion was that the three β subunits cycle between three conformational states, with different nucleotide-binding sites, in an energy-dependent reaction. Recent experiments, such as that depicted in Figure 16-31, have directly demonstrated that the γ subunit and attached ϵ subunit rotates relative to the fixed $(\alpha\beta)_3$ hexamer in discrete 120° steps, a reaction requiring hydrolysis of ATP. This

experiment established that rotation of the γ and ϵ subunits, normally powered by proton movement through the F_0 complex, drives the conformational changes that are required for binding of ADP and P_i, followed by synthesis and subsequent release of ATP.

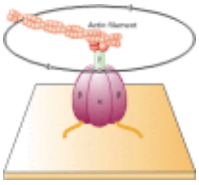


Figure 16-31

Demonstration that the γ subunit of the F_0 complex rotates relative to the $(\alpha\beta)_3$ hexamer in an energy-requiring step. F_1 complexes were engineered that contained β subunits with an additional His_6 sequence, which caused them (more...)

A simple calculation indicates that the passage of more than one proton is required to synthesize one molecule of ATP from ADP and P_i. Although the ΔG for this reaction under standard conditions is +7.3 kcal/mol, at the concentrations of reactants in the mitochondrion, ΔG is probably higher (+10 to +12 kcal/mol). We can calculate the amount of free energy released by the passage of 1 mol of protons down an electrochemical gradient of 220 mV (0.22 V) from the Nernst equation, setting $n = 1$ and measuring ΔE in volts:

$$\begin{aligned}\Delta G\ (\text{cal/mol}) = -n\mathcal{F}\Delta E &= -(23{,}062\ \text{cal} \cdot \text{V}^{-1} \cdot \text{mol}^{-1})\ \Delta E \\ &= (23{,}062\ \text{cal} \cdot \text{V}^{-1} \cdot \text{mol}^{-1})(0.22\ \text{V}) \\ &= -5{,}080\ \text{cal/mol, or } -5.1\ \text{kcal/mol}\end{aligned}$$

Thus, since just over 5 kcal/mol of free energy is made available, the passage of at least two, and more likely three or four, protons is essential for the synthesis of each molecule of ATP from ADP and P_i. This calculation has been confirmed by experimental data, which generally indicate that the passage of four protons through the F_0F_1 complex is coupled to a 120° rotation of the γ subunit and thus to the synthesis of one high-energy phosphate bond in ATP.

Transporters in the Inner Mitochondrial Membrane Are Powered by the Proton-Motive Force

The inner mitochondrial membrane contains a number of proteins that transport various metabolites into and out of the organelle, including pyruvate, malate, and the amino acids aspartate and glutamate. Two such proteins transport ADP and P_i from the cytosol to the mitochondrial matrix in exchange for ATP formed by oxidative phosphorylation inside the mitochondrion. The proton-motive force generated during electron transport is used to power this uphill exchange of ATP for ADP and P_i, which is carried out by an HPO_4^{2-}/OH^- antiporter (*phosphate transporter*) and an *ATP/ADP antiporter* (Figure 16-32).

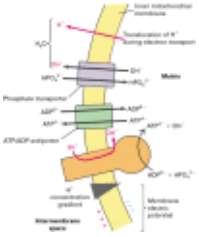


Figure 16-32

The phosphate and ATP/ADP transport system in the inner mitochondrial membrane. The coordinated action of two antiporters (purple and green) results in the uptake of one ADP^{3-} and one HPO_4^{2-} in exchange for one ATP^{4-},

powered by (more...)

The phosphate transporter catalyzes the import of one HPO_4^{2-} coupled to the export of one OH^-. Likewise, the ATP/ADP antiporter allows one molecule of ADP to enter only if one molecule of ATP exits simultaneously. The ATP/ ADP antiporter, a dimer of two 30,000-MW subunits, makes up 10 – 15 percent of the protein in the inner membrane, so it is one of the more abundant mitochondrial proteins. Functioning of the two antiporters together produces an influx of ADP and P_i and efflux of ATP and OH^-. Each OH^- transported outward combines with a proton, translocated during electron transport to the intermembrane space, to form H_2O. This drives the overall reaction in the direction of ATP export and ADP and P_i import.

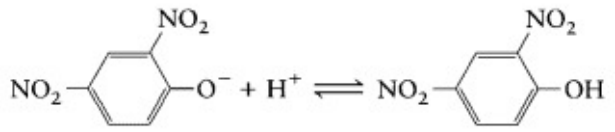
Because some of the protons translocated out of the mitochondrion during electron transport provide the power (by combining with the exported OH^-) for the ATP-ADP exchange, fewer protons are available for ATP synthesis. It is estimated that for every five protons translocated out, four are used to synthesize one ATP molecule and one is used to power the export of ATP from the mitochondrion in exchange for ADP and P_i. This expenditure of energy from the proton concentration gradient to export ATP from the mitochondrion in exchange for ADP and P_i ensures a high ratio of ATP to ADP in the cytosol, where the phosphoanhydride-bond energy of ATP is utilized to power many energy-requiring reactions.

Rate of Mitochondrial Oxidation Normally Depends on ADP Levels

If intact isolated mitochondria are provided with NADH (or $FADH_2$), O_2, and P_i, but not with ADP, the oxidation of NADH and the reduction of O_2 rapidly cease as the amount of endogenous ADP is depleted by ATP formation. If ADP then is added, the oxidation of NADH is rapidly restored. Thus mitochondria can oxidize $FADH_2$ and NADH only as long as there is a source of ADP and P_i to generate ATP. This phenomenon, termed *respiratory control,* illustrates how one key reactant can limit the rate of a complex set of interrelated reactions. Intact cells and tissues also employ respiratory control. Stimulation of a metabolic activity that utilizes ATP, such as muscle contraction, results in an increased level of cellular ADP; this, in turn, increases the rate of glucose breakdown in the glycolytic pathway and citric acid cycle, as discussed earlier, and the subsequent oxidation of the metabolic products in the mitochondrion.

The molecular nature of respiratory control is now well understood. Recall that the oxidation of NADH, succinate, or $FADH_2$ is *obligatorily* coupled to proton transport across the inner mitochondrial membrane. If the resulting proton-motive force is not dissipated by harnessing it to synthesize ATP from ADP and P_i (or for some other purpose), both the transmembrane proton concentration gradient and the membrane electric potential will increase to very high levels. NADH oxidation will eventually cease, because it will require too much energy to move additional protons across the inner membrane against the existing proton-motive force. Although the availability of ADP for ATP synthesis — that is, respiratory control — is the primary way that mitochondrial oxidation is regulated in intact cells, it is not the only way. For example, a rise in cytosolic Ca^{2+} ions, as occurs in muscle cells during contraction, also triggers an increase in mitochondrial oxidation and ATP production in many cells.

Certain poisons, called uncouplers, render the inner mitochondrial membrane permeable to protons. Uncouplers allow the oxidation of NADH and the reduction of O_2 to continue at high levels but do not permit ATP synthesis. In the uncoupler *2,4-dinitrophenol (DNP),* two electronwithdrawing nitro (NO_2) groups stabilize the negatively charged phenolate form:



Both the neutral and negatively charged forms of DNP are soluble in phospholipid membranes and in aqueous solution, so DNP can act as a proton shuttle. By transporting protons across the inner membrane into the matrix, DNP short-circuits both the transmembrane proton concentration gradient and the membrane electric potential, thereby dissipating the proton-motive force. Uncouplers such as DNP thus abolish ATP synthesis and overcome respiratory control, allowing NADH oxidation to occur regardless of the ADP level. The energy released by the oxidation of NADH in the presence of DNP is converted to heat.

Brown-Fat Mitochondria Contain an Uncoupler of Oxidative Phosphorylation

Brown-fat tissue, whose color is due to the presence of abundant mitochondria, is specialized for the generation of heat. In contrast, *white-fat tissue* is specialized for the storage of fat and contains relatively few mitochondria.

In the inner membrane of brown-fat mitochondria, a 33,000-MW inner-membrane protein called *thermogenin* functions as a natural uncoupler of oxidative phosphorylation. Thermogenin does not form a proton channel like the F_0 complex and typical channel proteins. Rather, thermogenin is a proton transporter whose amino acid sequence is similar to that of the mitochondrial ATP/ADP antiporter (see Figure 16-32); it functions at a rate that is characteristic of transporters, but is 1-million-fold slower than typical channel proteins (see Figure 15-3). Like other uncouplers, thermogenin dissipates the proton-motive force across the inner mitochondrial membrane, converting energy released by NADH oxidation to heat.

The amount of thermogenin is regulated depending on environmental conditions. For instance, during the adaptation of rats to cold, the ability of their tissues to generate heat (*thermogenesis*) is increased by the induction of thermogenin synthesis. In cold-adapted animals, thermogenin may constitute up 15 percent of the total protein in the inner mitochondrial membrane.

Adult humans have little brown fat, but human infants have a great deal. In the newborn, thermogenesis by brown-fat mitochondria is vital to survival, as it also is in hibernating mammals. In fur seals and other animals naturally acclimated to the cold, muscle-cell mitochondria contain thermogenin; as a result, much of the proton-motive force is used for generating heat, thereby maintaining body temperature.

SUMMARY

- The proton-motive force, a combination of a proton concentration (pH) gradient (exoplasmic face > cytosolic face) and an electric potential (negative cytosolic face), can be generated across the inner mitochondrial membrane, chloroplast thylakoid membrane, and bacterial plasma membrane. It is the energy source for ATP synthesis by the F_0F_1 complexes located in these membranes (see Figure 16-2).
- In the mitochondrion, the flow of electrons from NADH and $FADH_2$ to O_2 is coupled to the uphill transport of protons from the matrix across the inner membrane to the intermembrane space, generating the proton-motive force (pmf).
- The major components of the electron transport chain are four inner membrane multiprotein complexes: succinate-CoQ reductase, NADH-CoQ reductase, $CoQH_2$ – cytochrome *c* reductase, and cytochrome *c* oxidase. The last complex transfers electrons to O_2 to form H_2O.
- Electrons are transferred along the electron transport chain by the reversible reduction and oxidation of iron-sulfur clusters, ubiquinone (CoQ), cytochromes, and copper ions. Each carrier accepts an electron or electron pair from a carrier with a less positive reduction potential and transfers the electron to a carrier with a more positive reduction potential. Thus the reduction potentials of electron carriers favor unidirectional electron flow from NADH and $FADH_2$ to O_2 (see Figure 16-17).
- A total of 10 H^+ ions are translocated from the matrix across the inner membrane per electron pair flowing from NADH to O_2 (see Figure 16-19). Proton movement occurs at three points in the electron transport chain: the NADH-CoQ reductase (four H^+), $CoQH_2$ – cytochrome *c* reductase (four H^+), and cytochrome *c* oxidase (two H^+).
- CoQ functions as a lipid-soluble transporter of electrons and protons across the inner membrane. The Q cycle allows additional protons to be translocated per pair of electrons moving to cytochrome *c*.
- The multiprotein F_0F_1 complex catalyzes ATP synthesis as protons flow back through the inner membrane down their electrochemical proton gradient. F_0 is a transmembrane complex that forms a regulated H^+ channel. F_1 is tightly bound to F_0 and protrudes into the matrix; it contains three β subunits that are the sites of ATP synthesis (see Figure 16-28).
- Proton translocation through F_0 powers the rotation of the γ subunit of F_1, leading to changes in the conformation of the nucleotide-binding sites in the F_1 β subunits (see Figure 16-30). By means of this binding-change mechanism, the F_0F_1 complex harnesses the proton-motive force to power ATP synthesis.
- The F_0F_1 complexes in bacterial plasma membranes and chloroplast thylakoid membranes are very similar in structure to the mitochondrial F_0F_1complex.
- The proton-motive force also powers the uptake of P_i and ADP from the cytosol in

exchange for mitochondrial ATP and OH^- (see Figure 16-32). Import of ADP and P_i to the mitochondrion and the export of ATP from it coordinate and limit the rate of ATP synthesis to meet the cell's needs.

- Mitochondrial oxidation of NADH and the reduction of O_2 continue to proceed only if sufficient ADP is present. This phenomenon of respiratory control is an important, but not the only, mechanism for controlling oxidation and ATP synthesis in mitochondria.
- In brown fat, the inner mitochondrial membrane contains thermogenin, a proton transport protein that converts the proton-motive force into heat. Certain chemicals (e.g., DNP) have the same effect, uncoupling oxidative phosphorylation from electron transport.

Footnotes

* Note that the transmembrane electric potential that contributes to the proton-motive force and the resting electric potential across the plasma membrane, discussed in Chapter 15, are generated by fundamentally different mechanisms. The first results from the transport of H^+ ions *against* their concentration gradient powered by electron transport; the second results primarily from the movement of K^+ ions from the cytosol to the cell exterior, *down* their concentration gradient, through open potassium channels.

Bookshelf ID: NBK21528