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SnapShot: CRISPR-RNA-Guided Adaptive Immune Systems

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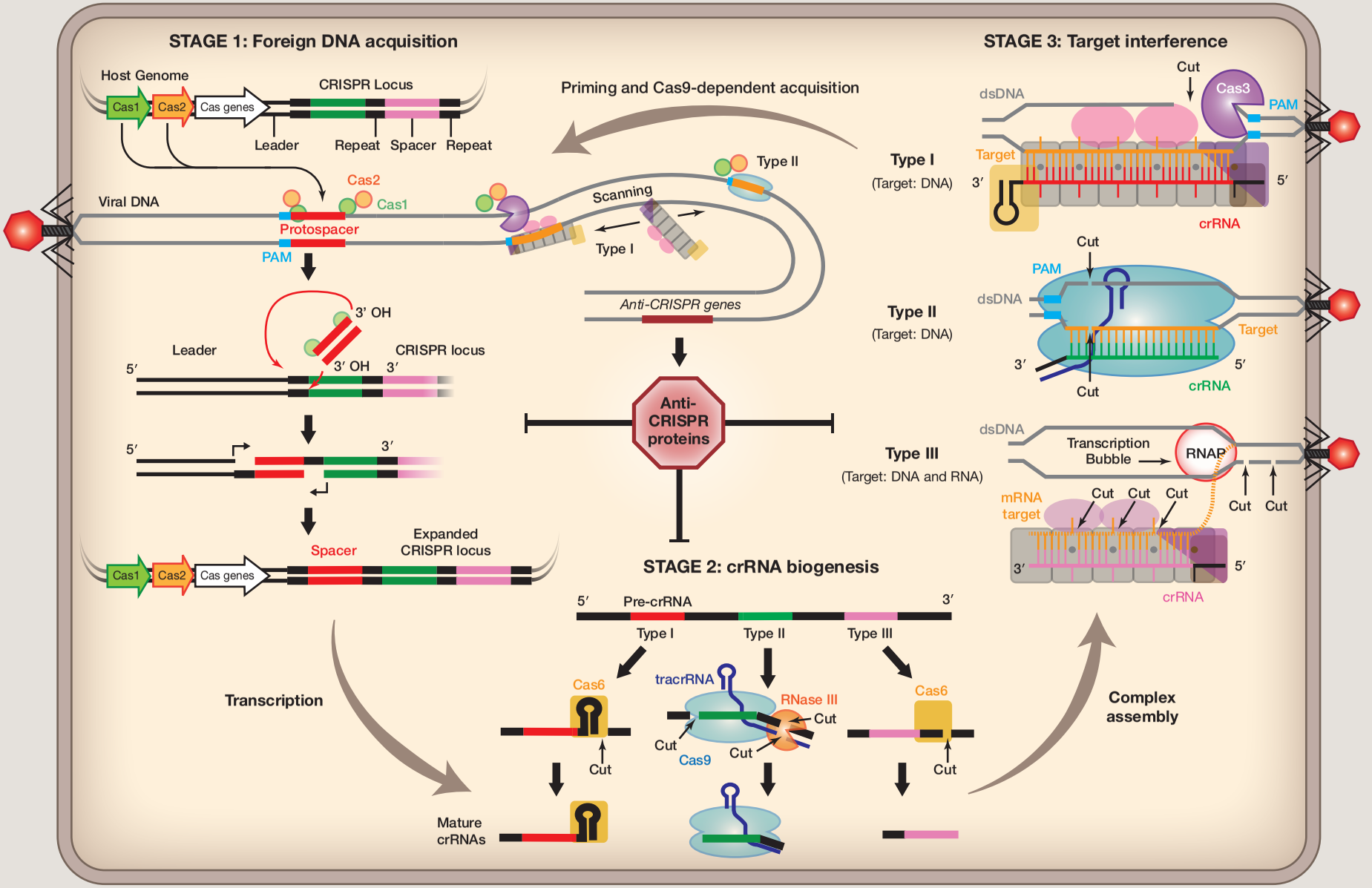
Cell



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Bacteria and archaea have evolved sophisticated adaptive immune systems that rely on CRISPR (*c*lustered *r*egularly *i*nterspaced *s*hort *p*alindromic *r*epeat) loci and a diverse cassette of CRISPR-associated (*cas*) genes (Sorek et al., 2013). CRISPR systems are classified into three main types (I–III) and at least eleven different subtypes (I-A to I-F, II-A to II-C, and III-A to III-B) (Makarova et al., 2011). Despite this diversity, all CRISPR-Cas immune systems operate through three main stages: acquisition, CRISPR RNA (crRNA) biogenesis, and target interference.

Stage 1. Foreign DNA Acquisition

Foreign nucleic acids are recognized by Cas proteins, and short fragments (30–50 base pairs) of invading DNA, called protospacers, are inserted into the host's CRISPR locus as spacer sequences, separated by repeat sequences. In type I and II systems, protospacers are selected from regions of invading DNA that are flanked by a 2–5 nucleotide (nt) motif called a PAM (*p*rotospacer *a*djacent *m*otif) (Sorek et al., 2013). Protospacers are generally incorporated at one end of the CRISPR locus, referred to as the leader, by a mechanism that involves Cas1, Cas2, and free 3′ hydroxyls on the protospacer (Nuñez et al., 2015). Protospacer integration is accompanied by the duplication of the leader-terminal repeat sequence, which may involve host polymerases and DNA repair machinery.

Stage 2. crRNA Biogenesis

CRISPR RNA biogenesis starts with transcription, followed by nucleolytic processing of the primary transcript (pre-crRNA) into a library of short CRISPR-derived RNAs (crRNAs) that each contains a sequence complementary to a previously encountered foreign DNA. The crRNA-guide sequence is flanked by regions of the adjacent repeats. In type I and III systems, the primary CRISPR transcript is processed by CRISPR-specific endoribonucleases (Cas6 or Cas5d) that cleave within the repeat sequence (Sorek et al., 2013). In many type I systems, the repeat sequences are palindromic and Cas6 remains stably associated with a stem loop on the 3′ end of the crRNA (Sorek et al., 2013). In type III systems, Cas6 transiently associates with the CRISPR RNA, and the 3′ end of the crRNA is further trimmed by unknown nucleases. CRISPR RNA processing in type II systems relies on a *trans*-acting crRNA (tracrRNA), which contains a sequence that is complementary to the repeat sequences (Jackson and Wiedenheft, 2015; Sorek et al., 2013). These double-stranded regions are processed by RNase III while in the presence of Cas9 (Sorek et al., 2013). In type II systems, both the tracrRNA and the crRNA are required for target interference (Sorek et al., 2013). The two RNAs from this system have been fused into a single-guide RNA (sgRNA), and Cas9 has become a powerful tool for targeted genome engineering in a wide variety of cell types and multicellular organisms (Hsu et al., 2014).

Stage 3. Target Interference

The mature crRNAs guide Cas proteins to complementary targets. Target sequences are degraded by dedicated Cas nucleases, but the mechanisms of target degradation are diverse (Jackson and Wiedenheft, 2015; Sorek et al., 2013). Type I and II systems both target dsDNA substrates that contain a PAM and a complementary protospacer sequence. Target cleavage in type II systems is performed by a single protein (Cas9) and two RNAs, whereas type I systems rely on multi-subunit surveillance complexes that bind dsDNA substrates and then recruit Cas3, a *trans*-acting nuclease that is often fused to an ATP-dependent helicase (Sorek et al., 2013). Like type I systems, type III systems also rely on multi-subunit complexes for target detection, but unlike the type I systems, these complexes exhibit endogenous nuclease activity that degrades complementary RNA and target DNA in a transcription-dependent manner (Samai et al., 2015). Type III systems do not rely on a PAM for target recognition; rather, base pairing that extends beyond the guide sequence and into the 5′ handle of the crRNA signals "self" (the CRISPR locus contains sequences that are complementary to the guide and the 5′ handle) and prevents target cleavage (Samai et al., 2015).

Closing the Loop

In type I systems, target binding by the surveillance complex results in Cas3-mediated target degradation (direct interference) or primed acquisition, which involves crRNA-guided recruitment of Cas3, Cas1, and Cas2 to foreign DNA and results in rapid acquisition of new spacers into the CRISPR (Datsenko et al., 2012; Sorek et al., 2013). While primed acquisition has not yet been observed in type II systems, Cas9 is required for proper protospacer selection, suggesting a functional link between the target interference and foreign DNA acquisition (Heler et al., 2015; Wei et al., 2015). Recently, diverse viral-encoded genes that produce proteins known as anti-CRISPRs have been shown to subvert CRISPR systems by interfering with each of the different stages (Bondy-Denomy et al., 2015).

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