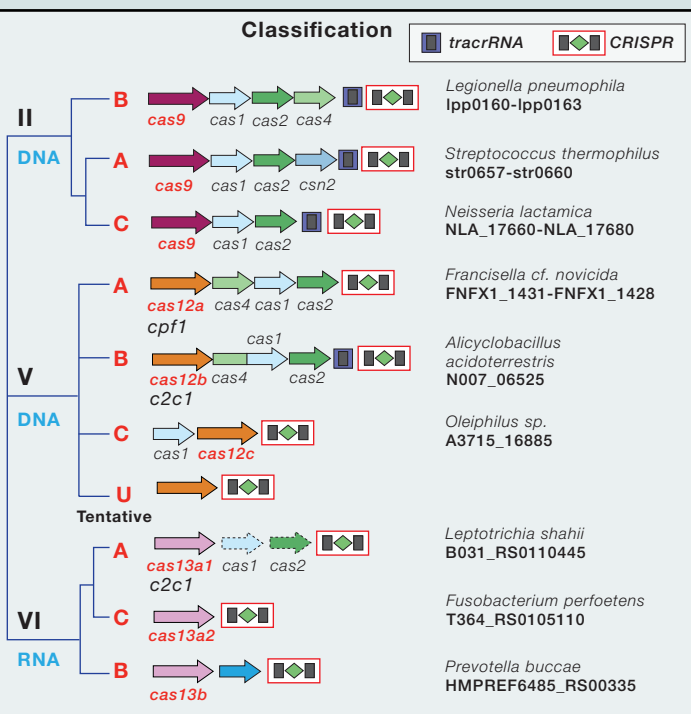
SnapShot: Class 2 CRISPR-Cas Systems

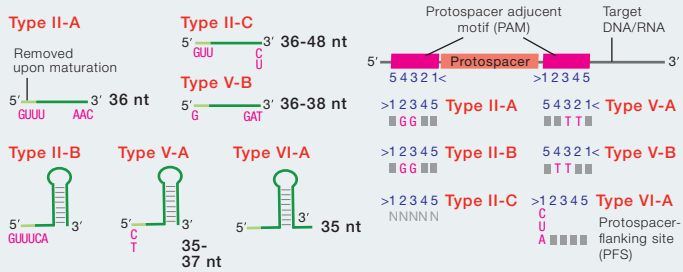
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Cell

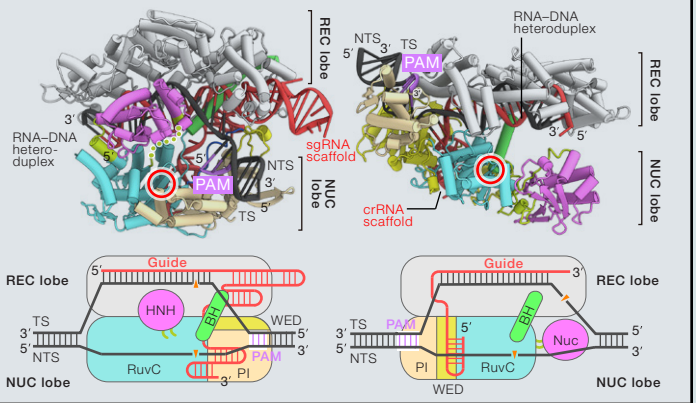
Classification



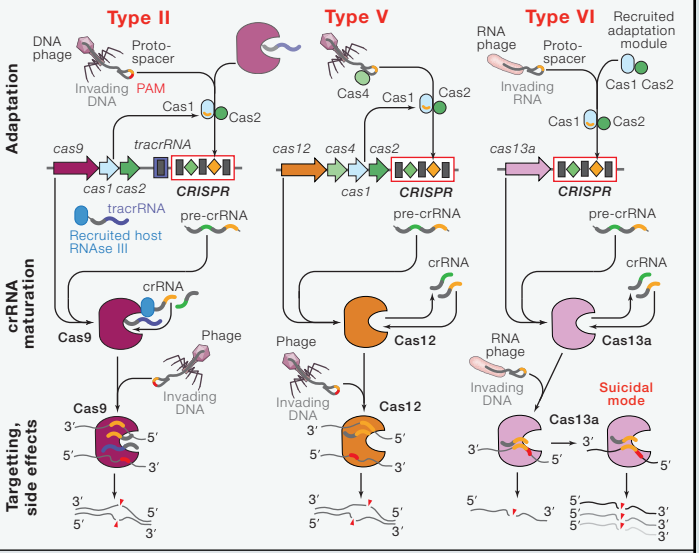
CRISPR and PAM features



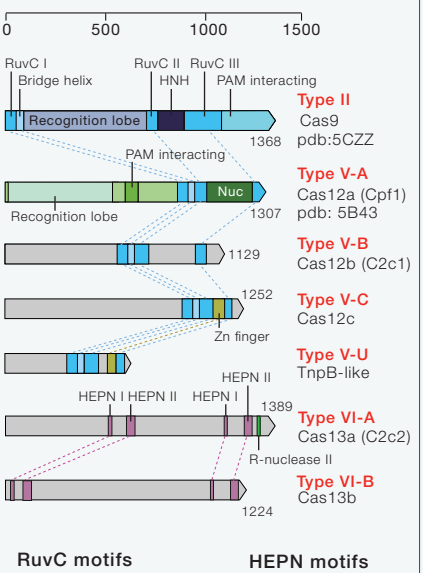
Effector protein structures



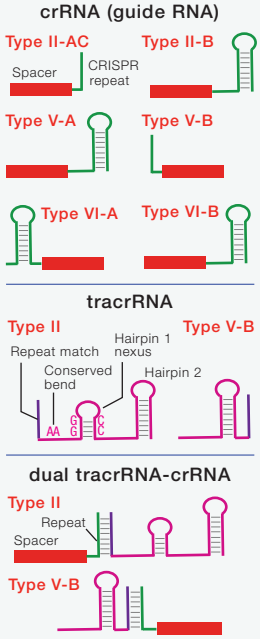
Mechanisms of action



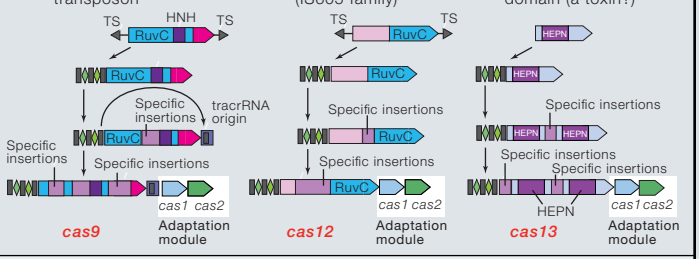
Effector protein domain organization



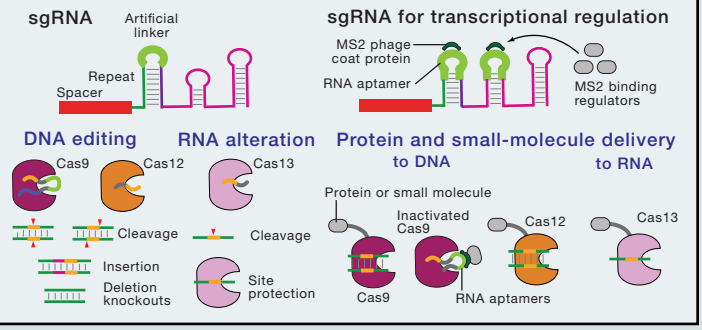
RNA components



Origin of class 2 systems



Guide RNA engineering and biotechnology applications



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SnapShot: Class 2 CRISPR-Cas Systems

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Classification

CRISPR (clustered regularly interspaced short palindromic repeat)-Cas systems are adaptive immune systems found in prokaryotes and are classified into two distinct classes based on effector protein organization. Class 1 CRISPR-Cas systems utilize multi-protein effector complexes, whereas class 2 CRISPR-Cas systems utilize single-protein effectors. Based on the distinct effector protein families, Class 2 systems are divided into three types and nine subtypes (Makarova et al., 2015; Shmakov et al., 2017). A tenth subtype (V-U) includes a number of putative systems for which an immune (or perhaps regulatory) function remains to be demonstrated (Shmakov et al., 2017). Class 2 systems represent about 10% of the CRISPR-Cas loci and are found in diverse bacterial phyla but virtually absent in archaea (Shmakov et al., 2017). In addition to the effector proteins, most of the Class 2 genomic loci encode adaptation module protein, Cas1 and Cas2, and accessory proteins, such as Cas4. Type II and type V-B loci also include tracrRNA (*trans*-activating CRISPR RNA), which is partially complementary to the repeats and is involved in CRISPR (cr) RNA processing and interference (Mohanraju et al., 2016). Some Class 2 systems, however, especially those of type VI, consist only of a CRISPR array and an effector protein.

CRISPR, RNA Components, and PAM

The direct repeats of Class 2 systems are either palindromic or non-palindromic. Type II and type V-B systems require tracrRNA for proper functioning, whereas type V-A systems use crRNA alone. The crRNA and tracrRNA can be artificially combined into a single guide RNA (sgRNA), which is widely used for Cas9-mediated genome editing applications (Komor et al., 2016). Typically, a protospacer adjacent motif (PAM), which varies between types and subtypes and can be located either up- or downstream of the protospacer, is required for interference by Class 2 systems (Leenay et al., 2016).

Domain Organization and Structure of the Effector Nucleases

There are three types of Class 2 effector proteins (Shmakov et al., 2017). Two of these, present in type II and type V systems, encompass a RuvC-like nuclease domain (RNAase H fold) that typically contains large domain insertions involved in crRNA and target binding. A second nuclease domain was identified in all type II systems (HNH family nuclease) and in type V-A (NUC domain, unrelated to the HNH domain, and so far unique among nucleases). An additional common feature between Cas9 and Cas12a is the arginine-rich bridge helix involved in tracrRNA binding. The crystal structures of the type II (Cas9) and type V-A (Cas12a) effectors revealed a shared bilobed shape but distinct PAM-interacting regions and recognition domains (Mohanraju et al., 2016). The effectors of type VI are unrelated to the type II and type V effectors and contain two ribonuclease domains of the HEPN superfamily (Shmakov et al., 2017); the structure of type VI effectors remains to be solved.

Mechanism of Action

As with other CRISPR systems, spacer acquisition requires Cas1 and Cas2 proteins, although involvement of both Cas9 and tracrRNA has been demonstrated for type II (Heler et al., 2015). The interference activity targets dsDNA in the case of type II and type V systems but targets ssRNA in the case of type VI systems (Abudayyeh et al., 2016). In the best-characterized type II system, tracrRNA is expressed and processed by RNase III, after which a complex of tracrRNA, Cas9, and RNase III processes the transcribed CRISPR array (pre-crRNA) (Mohanraju et al., 2016). The type II effector complex that consists of Cas9, tracrRNA, and guide crRNA cleaves the DNA target, producing blunt ends. The type V-A systems do not require tracrRNA and, using the crRNA alone as the guide, introduce a staggered cut into the target DNA (Zetsche et al., 2015). The type V-A effector, Cas12a, is responsible for the crRNA processing, in addition to the target cleavage (Fonfara et al., 2016). The type VI-A effector, Cas13a (C2c2), is an RNA-guided RNase. Once activated by the RNA target recognition, Cas13a becomes a non-specific RNase and apparently causes cell toxicity or death (Abudayyeh et al., 2016). Similar to Cas12a, the type VI-A effector, Cas13a, is responsible both for pre-crRNA processing and target RNA cleavage (East-Seletsky et al., 2016). Both HEPN domains of Cas13a are required for the latter but not the former activity.

Origins and Evolution

The effectors of type II and type V systems are homologous to RuvC-like nucleases encoded in (primarily) non-autonomous transposons, known as IscB and TnpB, respectively (Shmakov et al., 2017). Putative evolutionary intermediates have been discovered where TnpB-encoding genes are stably associated with CRISPR arrays. The current model of evolution posits that further insertions of additional domains and co-evolution with crRNA and possibly tracrRNA led to the emergence of large mutidomain effector proteins of types II and V. The type VI effectors could have evolved from HEPN domain-containing RNases that serve as accessory proteins in certain Class 1 CRISPR-Cas systems (Shmakov et al., 2017).

Applications

Following the original demonstration of Cas9-mediated genome editing, applications of CRISPR-Cas for genome regulation, monitoring, and beyond were subsequently developed (Komor et al., 2016). Conceptually, there are three major application tracks: (1) genome editing, including gene knockout, knockin, and mutation initiated with a dsDNA break made by a type II or type V effector nuclease; (2) gene regulation and delivery of various functional moieties (e.g., transcription factors) to unique sites in DNA using catalytically inactivated derivatives of the same nucleases; (3) targeting ssRNA for inactivation, editing, modification, or localization using a type VI effector (Cas13). The distinct features of the type V effectors compared to type II (e.g., autonomous crRNA processing, blunt cut, and orthogonal PAMs) provide versatility in the development of complementary applications, whereas type VI effectors potentially could have broad applications for RNA targeting.

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