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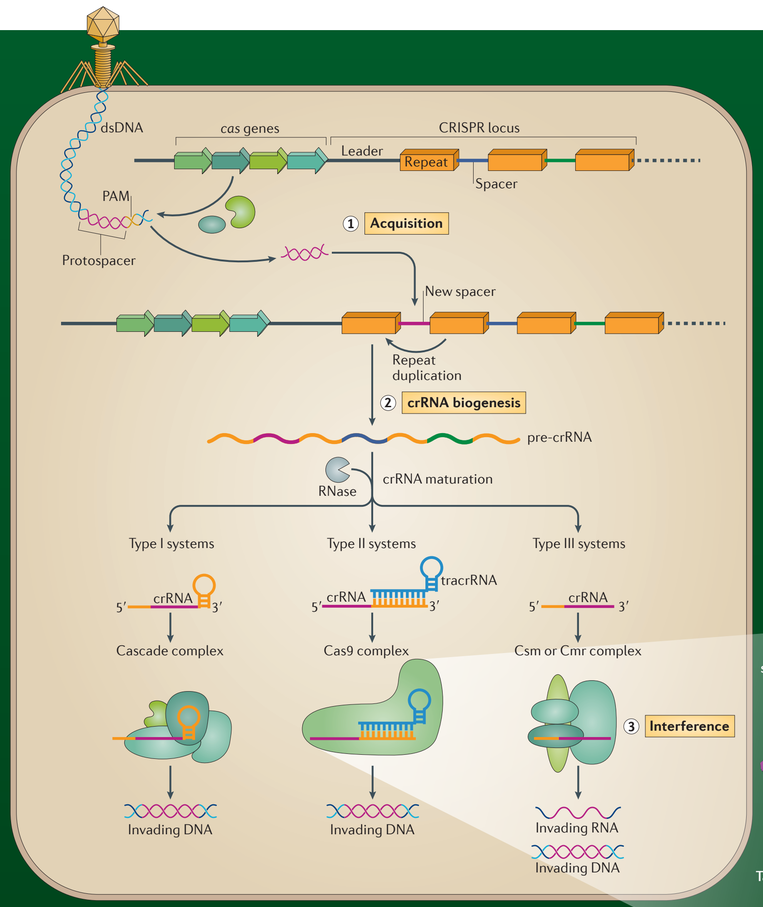
MICROBIOLOGY

CRISPR–Cas: extraordinary editing

CRISPR–Cas (clustered regularly interspaced short palindromic repeats–CRISPR-associated proteins) systems are genetic hallmarks of adaptive immunity in bacteria and archaea that have evolved to target and eliminate invading genetic elements such as viruses and plasmids. These systems consist of a *cas* gene cassette and a CRISPR array that encodes a series of direct repeats interspaced with short unique 'spacer' sequences that are derived from foreign DNA and therefore serve as genetic memory. Following transcription and maturation of the CRISPR locus, the CRISPR RNAs (crRNAs) that are generated function with the Cas proteins as a surveillance system for the detection of foreign DNA, which is then destroyed by RNA-guided interference. The nuclease Cas9 from the bacterial type II CRISPR–Cas system is transforming biological engineering as it is now used as an important tool for genome editing, on the basis of simple Watson–Crick base pairing. Its ease of use and efficiency have led to rapid adoption by many laboratories around the world for the editing of virtually any desired genome, including mammalian and microbial genomes. In addition, the system goes beyond genome editing and is also used for turning on and off gene expression and for imaging in live cells.

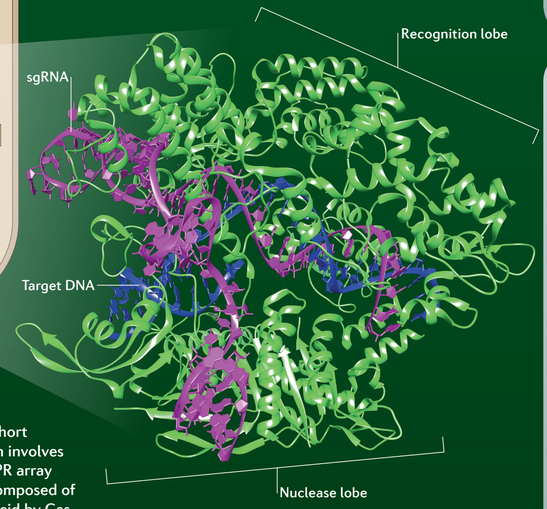


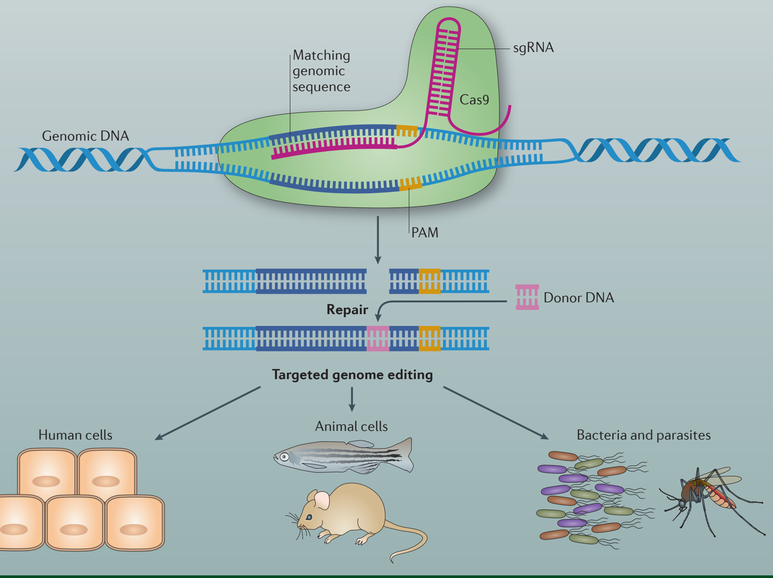
CRISPR–Cas: the basics

The first experimental evidence that CRISPR–Cas systems are involved in acquired immunity was provided by a study in 2007, which showed that infection of *Streptococcus thermophilus* with lytic phages provided subsequent immunity to the phage. The next year it was found that mature crRNAs function as guides in a complex with Cas proteins to interfere with viral DNA proliferation in bacteria. It is now known that adaptive immunity occurs in three stages: acquisition of a short sequence of the invading DNA (the protospacer) and its insertion into the CRISPR array as a spacer, which involves cleavage of the protospacer by Cas proteins and repeat duplication following recombination in the CRISPR array (step 1); transcription of the pre-crRNA, which undergoes processing to generate mature crRNAs, each composed of a repeat sequence and an invader-targeting spacer (step 2); crRNA-directed cleavage of foreign nucleic acid by Cas proteins at sites complementary to the crRNA spacer sequence, which is known as interference (step 3). There are three CRISPR–Cas system types (I, II and III) and each of these uses distinct molecular mechanisms for interference: the type I and type III systems use a large complex of Cas proteins for crRNA-guided targeting, whereas the type II system requires only the Cas9 protein for RNA-guided DNA recognition and cleavage, a property that has proved to be extremely useful for genome engineering applications.

Structure and function of the Cas9–sgRNA complex

Initial genetic studies showed that Cas9 is essential in viral defence, introduces DSBs into invading DNA and enables *in vivo* DNA targeting in bacteria. In 2012, Cas9 was shown to use two RNAs, the tracrRNA and crRNA, to direct DNA cleavage. Target recognition requires both base pairing to the crRNA sequence and the presence of a PAM that is adjacent to the targeted sequence. Importantly, the tracrRNA–crRNA hybrid can be engineered as an sgRNA that retains two crucial features: the 20-nucleotide sequence at the 5′ end that determines the DNA target site by Watson–Crick base-pairing and the double-stranded loop structure at the 3′ end that binds to Cas9. This discovery created a simple two-component system in which changes to the 20-nucleotide guide sequence of the sgRNA can programme Cas9 to target any DNA sequence of interest as long as it is adjacent to a PAM. In contrast to genome editing methods that require protein engineering for each DNA target site to be modified, the CRISPR–Cas9 system only requires a change in the guide RNA sequence to alter target specificity.





Genome engineering using Cas9–sgRNA

Owing to the ease of customization, CRISPR–Cas9 is now used to edit or modify the genomes of a vast number of cells and organisms, including bacteria, parasites, zebrafish, mice and human cells. When co-expressed with custom-designed sgRNAs in human cells (such as embryonic kidney cells, chronic myelogenous leukaemia cells or induced pluripotent stem cells), Cas9 generates DSBs in genomic DNA that are subsequently repaired by NHEJ to introduce gene disruptions or by HDR through the insertion of donor genetic sequences. Introducing DSBs at defined positions can generate human cell lines and primary cells carrying chromosomal translocations that resemble those that occur in lung cancer, acute myeloid leukaemia and Ewing's sarcoma. In addition, the targeting of several loci simultaneously with multiple sgRNAs has also been achieved, which is known as multiplexing. CRISPR-Cas9 has already been used to correct certain disease mutations, including those found in β-thalassaemia, tyrosinaemia and cystic fibrosis. CRISPR–Cas9 thus provides a robust and malleable tool to study genomic rearrangements, increases our understanding of the development of cancers and other diseases, and it has the potential to enable genetics-based therapeutics.

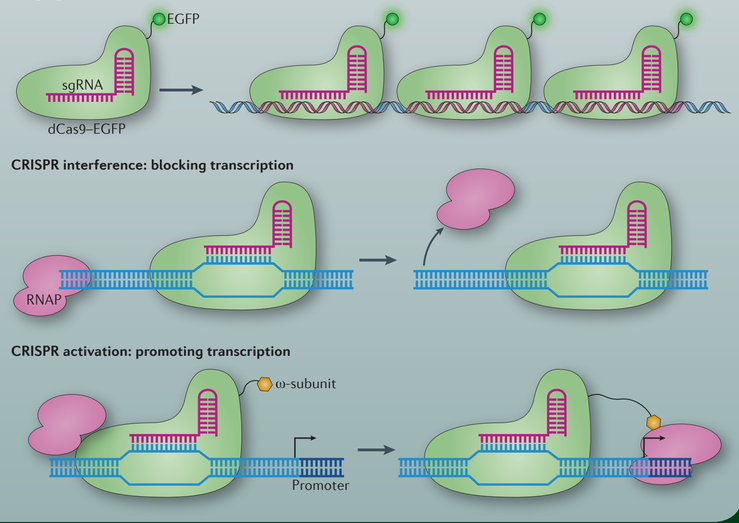
Other applications

Imaging in live cells

The ability to programme Cas9 to bind to any desired sequence can be exploited for the imaging of specific chromosomal positions in live cells. A modified Cas9 protein (dCas9), in which the nuclease domains are deactivated, is fused to a fluorescent protein (such as EGFP), and together with a specifically designed sgRNA, dCas9 can be used to image coding and non-coding DNA in living cells. This imaging tool has the potential to substantially improve the current technologies for studying conformational dynamics of native chromosomes in living cells. It may also be possible to couple fluorescent proteins or small molecules to the sgRNA, thereby providing an orthogonal strategy for multicolour imaging using Cas9.

Gene regulation

A key property of Cas9 is its ability to bind to DNA at sites defined by the guide RNA sequence and the PAM, which enables applications beyond permanent modification of DNA. In particular, dCas9 in combination with engineered sgRNAs has been repurposed for targeted gene regulation on a genome-wide scale. In a process known as CRISPRi, dCas9 can be used to block RNAP access to the DNA, and thereby reversibly repress transcription in bacteria and in human cells. Furthermore, by generating chimeric versions of dCas9 that are fused to regulatory domains (such as the RNAP ω-subunit), it has been possible to extend CRISPRi for efficient gene activation.



Abbreviations

Cas, CRISPR-associated proteins; CRISPR, clustered regularly interspaced short palindromic repeats; crRNAs, CRISPR RNAs; CRISPRi, CRISPR interference; dCas9, deactivated Cas9; DSBs, double-stranded breaks, dsDNA, double-stranded DNA; EGFP, enhanced green fluorescent protein; HDR, homology directed repair; NHEJ, non-homologous end joining; PAM, protospacer adjacent motif; pre-crRNA, precursor crRNA; RNAP, RNA polymerase; sgRNA, single-guide RNA; tracrRNA, transactivating crRNA.

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Competing interests statement

J.A.D. is an inventor of related patents and a co-founder of Caribou Biosciences and Editas Medicine, two companies that are commercializing CRISPR/Cas9 technology.

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Further reading

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