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Molecular mechanism of CRISPR

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The CRISPR-associated endonuclease Cas9 can be targeted to specific genomic loci by single guide RNAs (sgRNAs). We first solved the crystal structure of Streptococcus pyrogenes Cas9 (SpCas9) in complex with sgRNA and its target DNA at 2.5 A resolution. The structure revealed a bilobed architecture consisting of target recognition and nuclease lobes (Rec and Nuc lobes, respectively), accommodating the sgRNA:DNA heteroduplex in a positively-charged groove at their interface. While Rec lobe is essential for binding sgRNA and DNA, Nuc lobe contains the HNH and RuvC nuclease domains, which are properly located for cleavage of the complementary and noncomplemantary strands of the target DNA, respectively. Nuc lobe also contains a C-terminal domain responsible for the recognition of the protospacer adjacent motif (PAM). We further solved the crystal structure of more compact Staphyrococcus aureus Cas9 (SaCas9) and large Francisella novicida Cas9 (FnCas9) complexed with their guide RNAs and double-stranded target DNAs at 2.6 and 1.8 A resolutions, respectively. These high-resolution structures combined with functional analyses revealed the molecular mechanism of RNA-guided DNA targeting by Cas9, and uncovered the distinct mechanisms of PAM recognition. On the basis of the structures, we succeeded in changing the specificity of PAM recognition, which paves the way for rational design of new, versatile genome-editing technologies. Recently, we solved the crystal structure of type-V CRISPR, Cpf1 in complex with crRNA and target dsDNA. The structure explains striking similarity and major differences between Cas9 and Cpf1.

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