GENOMICS

New kid on the CRISPR block

The small, single RNA–guided nuclease Cpf1 is active in human cells.

Chances are that you have not heard of *Francisella novicida*, a Gram-negative pathogenic bacterium. Yet its defense system could be the basis for improved genome engineering, as Feng Zhang and his team from the Broad Institute of MIT and Harvard recently demonstrated.

In recent years, the bacterial adaptive immune system CRISPR-Cas9, in which RNA guides the Cas9 nuclease to its complementary DNA, has been applied to genome modification in many species. Most of this work is based on Cas9 from *Streptococcus pyogenes* (SpCas9), which, despite its successful use in this system, has some limitations. The SpCas9 protein is large, encoded by a >4-kilobase gene, and it requires the combination of an ~55-nucleotide trans-activating CRISPR RNA (tracrRNA) and an ~45-nucleotide guide RNA to recruit Cas9 and direct it to its DNA target next to a G-rich protospacer-adjacent motif (PAM).

Zhang and his team set out to characterize another CRISPR-Cas system that had recently been identified in several bacterial genomes, the 1.3-kb Cpf1 (CRISPR from *Prevotella* and *Francisella*). Working with Cpf1 from *F. novicida* in *Escherichia coli*, they demonstrated three advantages in addition to its small size: it does not require a tracrRNA and is recruited and targeted by a single guide RNA around 44 nucleotides in length; it cleaves about 20 bases away from a T-rich PAM, rather than adjacent to the PAM as Cas9 does; and it produces DNA double-strand breaks with several bases of 5′ overhang, in contrast to Cas9's blunt ends.

The big question was whether Cpf1 would be active in higher organisms. The team looked at Cpf1 proteins from 16 species and determined the PAM sequences of seven of them, which varied only in the number of T's per PAM. Outfitted with a nuclear localization signal, each Cpf1, together with a guide RNA targeting *DNMT1*, was transfected into human cells. Two showed efficient cleavage in multiple different cell lines.

Cpf1's small size and staggered cutting may allow for easy delivery to cells and effective integration of DNA inserts in their proper orientation. The fact that Cpf1 cleaves distant from its PAM could allow for multiple rounds of targeting with the same guide RNA. For example, Zhang's team envisions the introduction of an insertion or deletion in round one and homologous recombination in round two. Once issues such as off-target cleavage and efficacy are addressed, Cpf1 will be a welcome complement to Cas9.

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RESEARCH PAPERS

Zetsche, B. *et al.* Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell* **163**, 759–771 (2015).