GENOMICS

Spotlight on Cas12

A search for more type V Cas12 family members turns up unexpected functionality.

During the first wave of the CRISPR revolution, it was all about Cas9. In the CRISPR universe, Cas9 belongs to the class 2, type II systems, characterized by single-effector enzymes that are programmed via a short guide RNA complementary to a genomic target. Cas9 also needs a short tracrRNA in order to function. Cas9 binds adjacent to a PAM sequence on the target and introduces double-strand breaks via its two nuclease domains, HNH and RuvC.

But Cas9 is only one of myriad RNA-guided nucleases in bacterial genomes. Researchers such as Eugene Koonin from the US National Institutes of Health have been systematically mining prokaryotic genomes to find more CRISPR systems. Another member of the class 2 systems that gained traction as a research tool is the type V Cas12a effector, formerly known as Cpf1. It is smaller, does not need a tracrRNA, and, in addition to cleaving DNA via its RuvC domain, has RNase activity, which allows the processing of its own guide RNA array. Also, it recognizes a different PAM sequence, upstream of its target.

David Scott and colleagues from Arbor Biotechnologies, in collaboration with Koonin, decided to take a more in-depth look at the entire Cas12 family to find effectors with new functionality (Yan et al., 2019). “What motivates us is how there is still so much yet-to-be characterized CRISPR diversity in nature,” comments Scott.

They built and searched a database of almost 300,000 putative CRISPR–Cas elements for type V systems—nucleases with only one predicted RuvC nuclease domain. They discovered that the type V phylogenetic tree has three major branches: one with Cas12a, -c, -d, and -e; a second that includes Cas12b, -h, and -i; and a third with small effectors such as Cas12g. The important task was, of course, to ascertain functionality; the team used a negative selection screen in *Escherichia coli* to see which Cas12 subtypes could target and cleave DNA encoding essential genes in *E. coli* and thus reduce bacteria viability.

The researchers found a wide variety of DNA and RNA cleavage activities. Cas12g—the smallest of the Cas12 effectors, with ~800 amino acids—needed a ternary complex of target-specific guide RNA and tracrRNA to induce cleavage of single-stranded RNA. Once

activated, Cas12g nonspecifically cleaved single-stranded DNA and RNA in trans. Scott describes this discovery as particularly exciting, as it could have applications for RNA editing and RNA therapeutics. He points out that the collateral RNA cleavage could be exploited for the detection of viruses or the presence of certain mutations.

Cas12h and Cas12i did not need a tracrRNA for single-strand DNA cleavage and showed strong nickase activity on the DNA strand not paired with the guide RNA. “Cas12i has a unique biochemical profile with the potential to become a highly specific platform for therapeutic genome editing,” predicted Scott. Toward this goal, further development is needed, and Arbor Biotechnologies will submit the plasmids to Addgene to enable broad access.

In a parallel effort, Feng Zhang from the Broad Institute of MIT and Harvard, also in collaboration with Koonin, focused on Cas12b, a compact nuclease that requires both guide and tracrRNA and shows optimal cleavage activity at 48 °C, a temperature too high to use in mammalian cells (Strecker et al., 2019). They showed that, like for Cas9, guide and tracrRNA could be combined into a single guide RNA, but at physiological temperatures the enzyme showed mainly nicking activity, which limits its utility as a genome-editing tool. The researchers then introduced specific mutations in the area of the protein that binds the DNA to allow better access of the target DNA strand. They hypothesized that increased affinity would bring the target strand closer to the RuvC domain and lead to a cut in both DNA strands. The final engineered Cas12b was able to introduce double-strand breaks at 37 °C in human cells with high specificity.

With more tools available, the areas in mammalian genomes intractable for editing will decrease.

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Research papers

Yan, W. X. et al. Functionally diverse type V CRISPR–Cas systems. *Science* **363**, 88–91 (2019).
 Strecker, J. et al. Engineering of CRISPR–Cas12b for human genome editing. *Nat. Commun.* **10**, 212 (2019).

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