GENOMICS

CRISPR–Cas goes RNA

CasRx is a programmable CRISPR–Cas system that targets RNA for efficient knockdown and splicing modulation.

The CRISPR–Cas9 system has revolutionized genome engineering by allowing precisely targeted gene deletions and mutations down to the single-nucleotide level. Although RNA-targeting Cas9 enzymes have been described, their large size limits their use with convenient systems—such as adeno-associated virus (AAV)—for *in vivo* delivery. Seeking to expand the transcriptome engineering toolbox, Konermann *et al.* initiated a search for alternative, compact RNA-targeting CRISPR systems.

To identify CRISPR–Cas systems that might have been overlooked in prior efforts, Konermann *et al.* used a bioinformatics approach to screen prokaryotic genome sequences, first for putative CRISPR repeat arrays, then for nearby potential effector nucleases. The screen identified a novel family of compact single-subunit (type II) Cas enzymes containing two HEPN ribonuclease motifs characteristic of known Cas13 systems. The newly discovered Cas13d exhibited RNA-specific, guide-sequence-dependent cleavage activity and is small enough to be packaged within an AAV delivery vehicle along with a targeting array.

Konermann *et al.* then engineered optimized versions of several Cas13d orthologs for expression in human cells. Of those, CasRx, *Ruminococcus flavefaciens* Cas13d fused to a nuclear localization sequence, proved the most efficient at cleaving target sequences. CasRx was able to knock down the expression of coding and noncoding RNAs more selectively and efficiently than short-hairpin-RNA-based interference, which positions CasRx as a promising alternative tool for post-trancriptional gene silencing.

A modified CasRx unable to complete the cleavage reaction (dCasRx) retained interaction with its targets, suggesting that it could be leveraged to create a splice effector. Indeed, dCasRx targeted to RNA sequences that mediate splice selection by trans-acting factors altered splice isoform ratios in a predictable direction. Konermann *et al.* went on to demonstrate the power of their approach by delivering a programmed dCasRx to patient-derived cells and successfully modulating the ratio of tau protein isoforms, whose disregulation drives a number of neurodegenerative diseases.

The discovery of Cas13d expands the arsenal of genome editing tools, and it is likely that the ability to modulate splicing is but one of many uses it will be adapted for.

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RESEARCH PAPERS

Konermann, S. *et al.* Transcriptome engineering with RNA-targeting type VI-D CRISPR effectors. *Cell* https://dx.doi.org/10.1016/j.cell.2018.02.033 (2018).