GENOMICS

Keeping CRISPR in check

Phage biology yields useful anti-CRISPR proteins that may also lead to new CRISPR systems.

Nobody likes to stand idly by while being attacked and destroyed. Bacteria and phages are no exception. Bacteria have evolved CRISPR, an immune system that recognizes and eliminates invading viral nucleic acids, and phages have met the challenge with anti-CRISPR (Acr) proteins that inhibit the activity of the CRISPR systems.

The phage biologist Joseph Bondy-Denomy first discovered Acrs in 2013 while in Alan Davidson's lab at the University of Toronto. Having started his own lab at UCSF in 2015, he discovered and characterized the AcrIIA proteins that inactivate the widely used type II Cas9 protein, a single effector nuclease that is part of the CRISPR system from bacteria such as *Streptococcus pyogenes*. While now common as a tool in research labs for editing of eukaryotic cells, in bacteria type II CRISPR–Cas9 systems are dwarfed in number by type I systems, which are less attractive as genome-editing tools because they comprise multi-protein complexes. "We are interested in bacteriophage biology and what anti-CRISPRs do in the real world," explains Bondy-Denomy about their search for type I Acrs.

"The initial goal was to find new anti-CRISPRs, ones that are not homologous to known anti-CRISPRs, because they would also be more likely to inhibit new CRISPR systems," he outlines. Graduate student Adair Borges and undergraduate student Jenny Zhang carried out a bioinformatics search of *Pseudomonas aeruginosa* genomes, which carry type I CRISPR systems, for the presence of anti-CRISPR-associated (aca) genes. They found seven gene families in the vicinity of a known aca gene, some of which showed anti-CRISPR activity against type I-E and I-F CRISPR systems.

One of these genes, *AcrIF11*, turned out to be present in over 50 other bacterial species. The researchers are interested in phage biology, but they also saw the practical implications of their finding. "If we find new anti-CRISPRs in new bacterial and phage species, why not find ones that will be useful for people?" says Bondy-Denomy.

Nicole Marino, a postdoctoral fellow in the lab, decided to focus on finding Acrs against type V Cas12a (Cpf1), another widely used, single-effector genome-editing tool. She began her search in bacterial genomes that carry self-targeting spacers in the CRISPR locus of bacteria, based on a technique previously developed by Bondy-Denomy. These are indicative of the presence of Acrs, as otherwise the bacteria would self-destroy. One of the prominent candidates was *Moraxella*, which carries type I and type V CRISPR systems. Marino looked for homologs of genes neighboring *AcrIF11*, cloned all the genes within the same loci, and tested them for anti-CRISPR activity. The effort paid off and led them to three new AcrVA proteins (*Science* **362,** 240–242; 2018).

In an independent effort, Kyle Watters and other members of the group of Jennifer Doudna at UC Berkeley formalized this search for bacterial self-targeting genomes in a computational approach. Notably, their systematic search also led to *Moraxella* and three new Cas12a inhibitors, one of which, AcrVA1, was identical to the proteins Marino found (*Science* **362,** 236–239; 2018).

Both groups showed that AcrVA1 potently inhibited Cas12a in human cells and is thus poised to be a useful tool for regulation of Cas12a editing.

Practical applications aside, Bondy-Denomy is excited about the principle they discovered, which could help scientists access an abundance of anti-CRISPRs. In *Moraxella* the Acr genes against type I and type V CRISPRs are in close proximity in the same locus. "We have never seen Acrs that inhibit completely different CRISPR types right next to each other," marvels Bondy-Denomy. "Every gene in that region is potentially an anti-CRISPR against some CRISPR system." He predicts that knowing that these genes inhibit CRISPR, even if the targeted CRISPR system has not been identified yet, will lead to future anti-CRISPR discoveries in many other phage and bacterial species. He predicts that inhibitors for Cas13, for example, increasingly popular for RNA targeting, can be found in these anti-CRISPR loci.

Nicole Rusk

Published online: 30 October 2018
https://doi.org/10.1038/s41592-018-0207-8

Research papers
Marino, N. D. et al. Discovery of widespread type I and type V CRISPR-Cas inhibitors. *Science* **362**, 240–242 (2018).
Watters, K. E. et al. Systematic discovery of natural CRISPR-Cas12a inhibitors. *Science* **362**, 236–239 (2018).