PHAGE BIOLOGY

Giving CRISPR the slip

“the mechanism of protection is not sequence specific”

To proliferate in their bacterial hosts, phages must overcome several antiviral mechanisms, including the CRISPR–Cas (clustered regularly interspaced short palindromic repeats–CRISPR-associated proteins) adaptive immune system. A new study reports the first examples of phage-encoded genes that inactivate this system.

CRISPR loci consist of multiple repeat sequences that are separated by variable spacer sequences, which are often homologous to the protospacer segments of phage genomes or plasmids. The precursor transcript from CRISPR loci is processed into small CRISPR RNAs (crRNAs), which, together with Cas proteins, guide the recognition and cleavage of foreign

nucleic acid. To determine whether phages possess genes to counteract this system, the authors examined the type I–F CRISPR–Cas system of *Pseudomonas aeruginosa* str. PA14 using a collection of lysogenized strains, each containing a different prophage genome. To test whether the prophages expressed anti-CRISPR activity, the ability of three CRISPR-sensitive phages to form plaques on these strains was measured. In contrast to unlysogenized *P. aeruginosa* str. PA14, several lysogenized strains supported the production of robust plaques, indicating that the integrated prophage inactivated the CRISPR–Cas system, allowing the CRISPR-sensitive phage to replicate.

The anti-CRISPR prophages were closely related and contained a set of atypical genes at the same genomic position. The expression of any one of eight of these genes (encoding five distinct proteins) from an inducible, high-copy-number plasmid in the unlysogenized strain led to substantially higher plaquing efficiency for all three CRISPR-sensitive phages. Thus, despite the three phages having distinct protospacer targets, each of the eight anti-CRISPR genes provided protection, indicating that

the mechanism of protection is not sequence specific. Furthermore, expression of the anti-CRISPR genes did not perturb the biogenesis of crRNAs or Cas proteins, suggesting that the inhibitory activity is exerted after the formation of the crRNA–Cas complex. By altering the DNA sequence of anti-CRISPR gene 35 of phage JBD30, while maintaining the amino acid sequence of the encoded protein, the authors found that full anti-CRISPR activity was retained, indicative of protein- rather than RNA-based inhibition. Finally, the authors also showed that expression of gene 35 in a phage lacking anti-CRISPR activity was sufficient to confer CRISPR–Cas insensitivity.

This is the first study to demonstrate the existence of anti-CRISPR genes, but the authors propose that such genes are likely to be prevalent and might account for the persistence of phages despite the widespread nature of CRISPR loci in bacteria and archaea.

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