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Microbiology

Viruses use RNA decoys to thwart CRISPR defences

Carolyn Kraus & Erik J. Sontheimer

Bacteria and archaea are microorganisms that often use RNA-guided defences called CRISPR to destroy the genomes of viruses that infect them. It now emerges that viruses make RNAs that act as mimics to divert such defences. **See p.601**

Microorganisms such as bacteria and archaea flourish despite the ubiquitous presence of viruses, which are called bacteriophages or phages, that can infect them. This resilience is partly due to the evolution of anti-phage defence systems that thwart viral infection[1]. On page 601, Camara-Wilpert *et al*.[2] uncover a previously unknown strategy that viruses use to divert antiviral defences down a dead-end path.

Prominent among anti-phage systems are what are termed adaptive immune pathways. These depend on genetic sequences called CRISPRs, which produce CRISPR RNA (crRNA) sequences that act as guides to direct the destruction of corresponding phage genomes[3]. In an ancient arms race that continues to this day, phages have in turn evolved countermeasures, such as Acr proteins, which function as anti-CRISPRs by jamming the CRISPR machinery and subverting anti-phage defences[4].

CRISPR regions of the genome include arrays of unique 'spacer' sequences, which are derived from the genomes of previously encountered phages. These spacers are separated from each other by short repeat sequences (Fig. 1a). The crRNAs produced from these CRISPR sites consist of a single spacer sequence flanked on one or both sides by portions of the repeats. CRISPR sites are generally accompanied in adjacent genome sequences by genes encoding Cas proteins, which assemble into crRNA-guided complexes that home in on matching sequences in the genomes of attacking phages.

Many categories of CRISPR–Cas systems (types I–VI) have been defined, and nearly all have been found to be susceptible to inhibition by more than one Acr protein. At least 100 Acr families have been identified[5], and these have a remarkable diversity in their structures, mechanisms and specificities for the subsets of CRISPR systems that they inhibit[6]. Most Acrs investigated thus far recognize and bind to Cas proteins and interfere with their interactions, structural transitions or other biochemical activities. Phages have been known to co-opt entire CRISPR–Cas systems for their own purposes, such as interference with other viruses during the infection of a microbe by more than one competing phage[7].

Occasionally, other CRISPR-like sequences known as solitary repeat units (SRUs) — each with only one spacer and one repeat — can be found in the genomes of phages and other mobile genetic elements (MGEs), such as a type of circular DNA called a plasmid. Strangely, unlike most viral CRISPR arrays, these SRUs are generally not accompanied by genes encoding Cas proteins. Camara-Wilpert and colleagues set out to explore the roles of these SRUs and test the hypothesis that they have anti-CRISPR functions.

To determine whether SRUs can protect phages from CRISPR-mediated interference, the authors identified an SRU with similarities to the crRNAs encoded by their chosen host bacterial strain and incorporated the SRU into that strain of bacterium. After finding that the SRU expressed small crRNA-like RNAs, the authors infected bacteria with a phage that is normally targeted and suppressed by the host bacterium's type I-F CRISPR–Cas system. Importantly, the authors found that, relative to their non-SRU-containing counterparts, SRU-expressing bacteria were much more susceptible to phage infection and

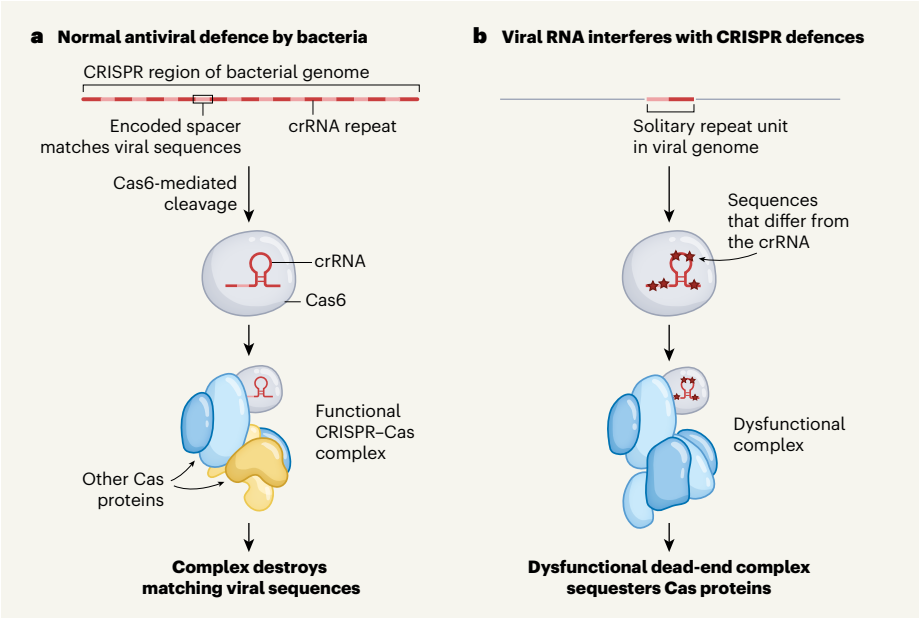

Figure 1 | How viruses combat a bacterial defence. a, Some bacteria use a type of defence that depends on what are called CRISPR sequences, encoded in the bacterial genome. These contain repeat sequences and spacer sequences that correspond to CRISPR RNAs (crRNAs), which match viral sequences. These sequences are cleaved by the enzyme Cas6 and form a complex with other Cas proteins that can destroy viral genomes. **b**, Camara-Wilpert *et al*.[2] reveal that some viruses can thwart such antiviral defences by encoding sequences called solitary repeat units that are similar to, but distinct from, segments of functional CRISPR sequences. The solitary repeat unit is cleaved by Cas6 and forms a dysfunctional complex with a subset of the usual Cas proteins.

virus-mediated bacterial destruction.

This result strongly indicates that SRUs from phages and MGEs can encode RNA anti-CRISPRs (termed Racrs) that act as inhibitors of CRISPR-mediated defences. The Racr examined by the authors — called RacrIF1 — bound specifically to a protein component (Cas6f) of the bacterial host's CRISPR–Cas machinery, and mutations in *racrIF1* that prevented Cas6f binding to RacrIF1 RNA undid its anti-CRISPR activity. Further biochemical analysis of the complex of Racr1F1 and Cas6f revealed the presence of other Cas proteins, albeit not the full cohort that normally functions in crRNA-directed CRISPR interference. The authors propose that RacrIF1 effectively functions as a crRNA mimic: its binding to Cas6f leads to the formation of an aberrant CRISPR complex that is non-functional, thereby sequestering Cas proteins to prevent their participation in anti-phage defence (Fig. 1b).

Are such crRNA mimics a widespread phenomenon corresponding to distinct CRISPR–Cas machineries — similar to their Acr protein counterparts — or are they unique to type I systems? To address this, the authors conducted computational searches that identified numerous SRUs in phages and other MGEs. Most of the candidate Racrs exhibited clear similarities to crRNAs harboured by the hosts they can infect, and examples were found for nearly every type of CRISPR–Cas system. These results strongly indicate that Racrs are a common, and previously underappreciated, mode of CRISPR counterdefence. Similar to the genes encoding Acr proteins, Racr-encoding SRUs are often found clustered with other anti-defence genes — consistent with their proposed function.

Two other studies also analysed crRNA-like small RNAs, including Cas-regulating RNAs, that modulate Cas protein expression and can detect Acr inhibition[8,9]. Some crRNA-like small RNAs were even noted as viral elements clustered with other genes encoding Acrs[9]. A broader picture emerges in which both bacteria and phages have co-opted and mimicked crRNAs for uses beyond CRISPR interference itself, and sometimes in direct opposition to it.

"The authors' results have extended the realm of anti-CRISPRs into the RNA world."

The authors' results have extended the realm of anti-CRISPRs into the RNA world, reflecting biotechnological approaches to restrain CRISPR–Cas9 genome editing[10] (in that case using RNAs as inhibitors rather than as molecular mimics). If the diversity of protein-based Acrs is any indication, Racrs might soon belong to a broader class of CRISPR inhibitors that exploit strategies beyond crRNA mimicry, and that provide further understanding of CRISPR interference mechanisms as well as insights into phage–host interactions and their co-evolution.

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