Phages' box of tricks for CRISPR

To sustain infection, phages must overcome bacterial defence mechanisms, such as the CRISPR–Cas adaptive immunity system that uses sequence memory to target invading DNA. Accordingly, several phage-encoded proteins that inhibit CRISPR–Cas activity have recently come to light. Now, a new study characterizes three of these anti-CRISPR proteins to provide the first mechanistic insights into phage evasion of CRISPR–Cas immunity.

Bondy-Denomy *et al.* purified AcrF1, AcrF2 and AcrF3, which they had previously identified as inhibitors of the type I-F CRISPR–Cas system in *Pseudomonas aeruginosa*. In this system, the Csy complex (comprising Csy1, Csy2, Csy4 and six copies of Csy3, together with a CRISPR RNA (crRNA)) recognizes invading DNA, which is then degraded by the Cas3 nuclease. Using size-exclusion chromatography (SEC), the authors showed that AcrF1 and AcrF2 both interact with the Csy complex, whereas AcrF3 interacts with Cas3. Furthermore, electrophoretic mobility shift assays showed that AcrF1 and AcrF2 block the binding of the Csy complex to the target DNA, whereas AcrF3 blocks the binding of Cas3 to the target-bound Csy complex.

To see whether these *in vitro* findings were representative of anti-CRISPR activity *in vivo*, the authors studied host–phage interactions in *P. aeruginosa*. First, they showed that overexpression of Csy components — to dilute interactions with anti-CRISPR proteins — reduced the viability of phage encoding AcrF1 or AcrF2, but not AcrF3, which confirmed that the Csy complex has direct roles in the anti-CRISPR mechanisms of AcrF1 and AcrF2. Next, the role of Cas3 was examined by exploiting a phenomenon in which the loss of Cas3 activity converts the Csy complex into a transcriptional repressor, owing to the stable physical interaction with target DNA that is formed in the absence of degradative activity. To monitor transcriptional repression resulting from Cas3

inhibition, the authors used an endogenous reporter gene (*phzM*) that is required for the production of the blue-green pigment pyocyanin. *phzM* was transcriptionally repressed in the presence of AcrF3, but not AcrF1 or AcrF2, which confirmed the *in vitro* data supporting a direct interaction between AcrF3 and Cas3.

Together, these *in vitro* and *in vivo* experiments clearly showed that AcrF3 has a distinct anti-CRISPR mechanism to AcrF1 and AcrF2. To address whether AcrF1 and AcrF2 inhibit the Csy complex by overlapping or independent mechanisms, the authors used SEC to identify the Csy components targeted, finding that AcrF1 interacts with the Csy1–Csy2 heterodimer and that AcrF2 interacts with the Csy3 hexamer. Assessing binding stoichiometry using protein gel electrophoresis suggested that each AcrF1 molecule binds to two Csy3 subunits, perhaps at Csy3–Csy3 interfaces.

Finally, the authors investigated whether these different targets within the Csy complex correspond to alternative mechanisms of inhibition. Competition assays suggested that AcrF2 sterically blocks the interaction between the 5' end of the crRNA, where Csy1–Csy2 is located, and the target DNA, whereas AcrF1 binding to Csy3 represents an allosteric mechanism that blocks interactions between the Csy complex and the full length of the target DNA.

These diverse mechanisms of anti-CRISPR activity are the legacy of a virus–host arms race that has deep evolutionary roots, which has probably given rise to additional mechanisms that await discovery. It is hoped that this diversity will inspire advances in biotechnological applications of CRISPR–Cas systems.

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