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BACTERIAL PHYSIOLOGY

It's never too late for CRISPR RNases

> type III-A CRISPR – Cas systems use three lines of defence to prevent the replication of invading phages

Several types of CRISPR–Cas adaptive immune system have been described, each with a unique mechanism and protein composition. DNA cleavage by type III-A CRISPR–Cas systems relies on a complex known as Cas10–Csm and requires transcription of the target DNA. Type III-A CRISPR–Cas systems are also capable of degrading RNA, although why they should have an RNase function is not known. Jiang *et al.* now show that some phage targets can delay the transcription-dependent DNA cleavage mechanism of type III-A CRISPR–Cas systems but that CRISPR RNases prevent the replication of these phages.

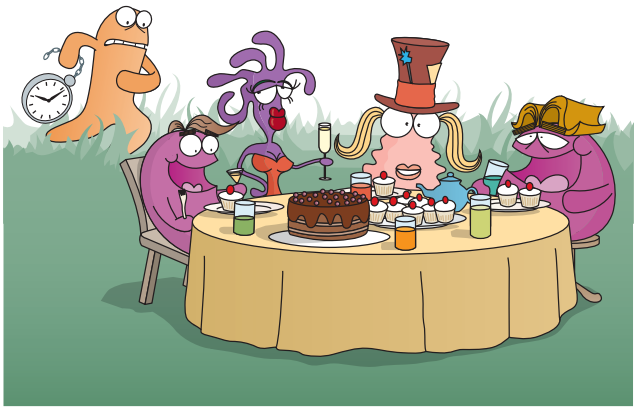
Most phages have a life cycle that includes early and late stages of gene expression, and sequences in both early- and late-stage genes can be acquired as targets by CRISPR–Cas systems. As DNA cleavage by type III-A CRISPR–Cas systems requires transcription of the target, the authors wondered whether the timing of gene expression would affect cleavage of target DNA by these systems. They found that the type III-A CRISPR–Cas system in *Staphylococcus epidermidis* was similarly effective against both early- and late-stage phage targets in wild-type cells. However, mutations that eliminated the RNase activity of CRISPR–Cas resulted in a loss of antiphage immunity for late-stage, but not early-stage, targets, which suggests that the RNase activity of type III-A CRISPR–Cas systems is only required for late-stage targets. The authors propose that transcription of early-stage genes enables the transcription-dependent DNA cleavage mechanism of type III-A CRISPR–Cas systems to degrade phage genomes before replication of these genomes can occur, whereas transcriptional delay enables late-stage targets to evade DNA cleavage during the early stage of the phage life cycle. To overcome this evasion, which would otherwise permit the replication of the phage genome during the early stage of the life cycle, type III-A CRISPR–Cas systems use RNases to degrade phage mRNA transcripts.

The RNase activity of type III-A CRISPR–Cas systems is mediated by two RNases, Csm3 and Csm6. The authors found that loss-of-function of either Csm3 or Csm6 alone had little or no effect on antiphage immunity, which suggests a redundancy between the two RNases so that either Csm3 or Csm6 is sufficient for the degradation of late-stage targets. However, Csm6 degraded long stretches of DNA up to at least 1 kb either side of the target, whereas cleavage by Csm3 was specific to the target.

Csm3 had previously been shown to require target-binding for its RNase activity, but an *in vitro* assay showed that the RNase activity of Csm6 is sequence independent, which raised the possibility of an additional role for Csm6 in targeting phages with escape mutations that would otherwise evade CRISPR–Cas immunity. Testing the efficacy of Csm3 or Csm6 loss-of-function mutants on phage targets with escape mutations showed that Csm6, but not Csm3, can inhibit the replication of phages with up to four escape mutations.

Together, the findings of the study show that type III-A CRISPR–Cas systems use three lines of defence to prevent the replication of invading phages: DNA cleavage by Cas10 for all targets, mRNA cleavage by Csm3 and Csm6 for late-stage targets, and mRNA cleavage by Csm6 for targets with escape mutations.

Naomi Attar



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ORIGINAL ARTICLE Jiang, W. *et al.* Degradation of phage transcripts by CRISPR-associated RNases enables type III CRISPR-Cas immunity. *Cell* http://dx.doi.org/10.1016/j.cell.2015.12.053 (2016)