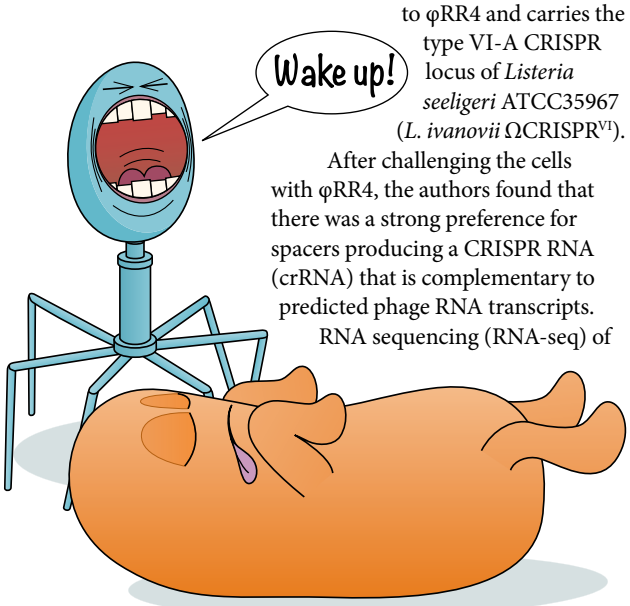
BACTERIAL PHYSIOLOGY

Playing dead during phage infection

> the trans-RNA degradation activity of Cas13 provides broad immunity against DNA phages by inducing a dormant state in infected cells

Bacteria and archaea have a variety of CRISPR–Cas systems that confer sequence-specific protection against invading plasmids and phages. Uniquely, the type VI effector nuclease Cas13 targets RNA instead of DNA, and catalyses both sequence-specific cis- and non-specific trans-RNA cleavage. Previous studies observed that type VI spacer sequences exclusively align with double-stranded DNA phages; however, the mechanism by which Cas13 uses its RNA cleavage activity to protect against DNA phages is not understood. Now, Meeske, Nakandakari-Higa and Marraffini show that trans-cleavage of both viral and host transcripts induces a state of dormancy in bacteria, which inhibits viral replication and the spread of CRISPR-resistant phages.

First, the authors investigated the ability of Cas13 to provide defence against the DNA phage φRR4 in *Listeria ivanovii* by introducing a spacer library of φRR4 genes into an engineered strain that is susceptible to φRR4 and carries the type VI-A CRISPR locus of *Listeria seeligeri* ATCC35967 (*L. ivanovii* ΩCRISPR^{VI}).



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After challenging the cells with φRR4, the authors found that there was a strong preference for spacers producing a CRISPR RNA (crRNA) that is complementary to predicted phage RNA transcripts.

RNA sequencing (RNA-seq) of φRR4-infected cells found a marked correlation between protospacer transcription and protection conferred by the spacer. Indeed, the presence of one of three spacers significantly reduced φRR4 replication. Together, these results suggested that Cas13 targets phage transcripts to block infection; however, the authors noticed that the function of the cis-targeted transcript appeared irrelevant and did not affect the potency of the response.

The authors hypothesized that Cas13-mediated trans-RNA degradation of both host and viral transcripts is important for type VI immunity. RNA-seq and global mapping of RNA 5′ ends showed substantial degradation of host transcripts and early and late φRR4 transcripts during infection. Moreover, degradation of host transcripts was found when the expression of *cas13* was induced without infection.

Previous observations of bacterial growth defects during type VI immunity against plasmids suggested that host transcript degradation could shift infected cells into a state of dormancy, thus preventing phage propagation. In *L. seeligeri* ATCC35967, wild-type (but not ΔCRISPR) cells had an arrested growth phenotype after *cas13a* induction, which recovered once induction was stopped. These cells were viable, suggesting that Cas13a-induced dormancy is reversible. Furthermore, these dormant cells were resistant to transient exposures of bactericidal antibiotics that target growing cells. Moreover, cytological assays suggested that *L. ivanovii* ΩCRISPR^{VI} cells entered into dormancy and remained viable after φRR4 infection. These results suggest that when Cas13 is activated by a target RNA, its RNase activity promotes a dormant state in the host, which is maintained in infected cells owing to the continued synthesis of protospacer RNA.

Next, the authors investigated whether the dormant state provides herd immunity to uninfected cells. Wild-type *L. ivanovii* RR3 (φRR4-sensitive) cells were protected from infection when co-cultured with *L. ivanovii* ΩCRISPR^{VI} cells that encode a φRR4-specific spacer. The authors transformed a plasmid that carries a target into *L. ivanovii* ΩCRISPR^{VI} cells (which carries a plasmid-targeting spacer, but not phage-targeting spacers). Pre-induction of *cas13a* using an inducible promoter resulted in an ~6.7-fold reduction in φRR4 infection efficiency. In addition, the plasmid-targeting strain had a survival advantage after 7 hours of infection. The observation that a plasmid-activated type VI system protected against phage infection suggested that the system can provide broad, non-specific immunity. Subsequent experiments showed that phages harbouring escape mutations could be inhibited if the system was first activated by a wild-type phage, thus averting the rise of CRISPR-resistant phages.

Altogether, these observations support a model in which the trans-RNA degradation activity of Cas13 provides broad immunity against DNA phages by inducing a dormant state in infected cells, thus thwarting phage replication and a viral epidemic.

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