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MICROBIOLOGY

Viruses cooperate to defeat bacteria

It emerges that viruses called phages, which infect bacteria, can suppress the bacterial immune system during an initial wave of unsuccessful infection, enabling subsequent viral infection to succeed.

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Bacteria and the viruses that infect them, known as phages, are engaged in a constant arms race. Bacteria continually evolve new mechanisms of resistance against viruses, while phages evolve countermeasures to overcome these defence mechanisms. Writing in *Cell*, Borges *et al.*[1] and Landsberger *et al.*[2] reveal how phages can 'collaborate' to shut down the bacterial immune system and achieve a successful infection. Although an initial viral attempt at infection fails, this enables a subsequent phage infection to be successful. This example of 'cooperation' between genetically identical individuals of a viral population illuminates a previously unknown group strategy of phages, and provides an interesting example of viral 'altruism'.

The bacterial anti-phage defence system called CRISPR–Cas recognizes and targets foreign nucleic acids in a sequence-specific manner[3]. To block CRISPR–Cas defences, phages express genes that encode proteins that inhibit the function of the CRISPR–Cas machinery[4,5]. Because these anti-CRISPR proteins are encoded in the phage genome (Fig. 1), a phage must enter a bacterial host cell and begin to express them to mount a counter-attack. Yet this raises a conundrum. CRISPR–Cas defences can attack phage DNA as soon as it enters a bacterial cell[6], before the phage gets a chance to express and use its anti-CRISPR proteins. So what purpose do anti-CRISPR proteins serve if the help they provide is likely to arrive too late for the individual virus that expresses them?

To investigate this, Borges, Landsberger and their respective colleagues studied the bacterium *Pseudomonas aeruginosa* and examined viral anti-CRISPR proteins that target a version of the bacterial CRISPR–Cas system called type I-F. Both groups observed that the initial ratio between the number of phages and the number of bacterial host cells, known as the multiplicity of infection (MOI), could be used to predict whether phage infection would be successful. When the authors tested bacteria that lacked CRISPR–Cas immunity, even a low dose of phages resulted in successful viral propagation that caused the eventual demise of the bacterial culture. However, for bacteria and phage populations encoding CRISPRs and anti-CRISPRs, respectively, phages managed to replicate only if the initial MOI was high.

The two groups followed different strategies to determine why the initial ratio of phages to bacteria is important for the success of phage propagation. Borges and colleagues used a genetic approach to test whether the inactivation of bacterial defences was due to the presence of anti-CRISPR proteins from more than one phage in the same host cell. The authors engineered a phage that was incapable of replication but did express anti-CRISPR proteins. By mixing these engineered phages with wild-type ones, the authors showed that the presence of the engineered phages could enable a wild-type phage infection to succeed even at a low MOI that would normally fail.

Landsberger and colleagues, by contrast, created a mathematical model to investigate the observed MOI dependence of phage infections. They could recapitulate their experimental findings only by using a model simulation in which bacteria enter an immunosuppressed state after surviving an initial infection by a phage that expressed anti-CRISPR proteins.

Both groups' results suggest that the first wave of phages that attempt infection succumb to CRISPR–Cas defences but manage to deliver anti-CRISPR proteins that immunosuppress the bacterial cell. This initial attack paves the way for a second wave of phages to successfully infect the now-defenceless bacterium. Having a high MOI increases the probability that a second wave of phage infection will occur.

The authors of these two studies validated this hypothesis in different ways. Landsberger and colleagues infected a bacterial population with phages encoding anti-CRISPR proteins and then introduced a type of circular DNA called a plasmid into the bacteria. Plasmid DNA would normally be targeted by the bacterial immune system, but the earlier phage

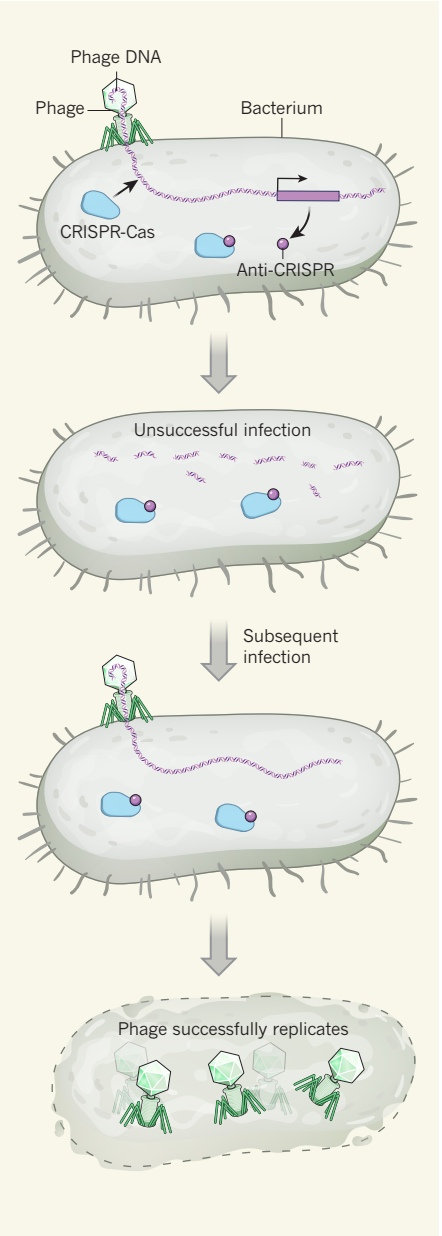

Figure 1 | A viral mechanism to thwart bacterial defences. The bacterial protein and RNA complex termed CRISPR–Cas recognizes and destroys foreign DNA, such as that of invading bacteria-infecting viruses called phages, in a sequence-specific manner[3]. Some phages encode anti-CRISPR proteins that bind to CRISPR–Cas and inhibit its function[4,5]. However, how such proteins aid viruses has been unclear, given that an initial infecting phage is probably targeted and destroyed by CRISPR–Cas, even if the phage expresses anti-CRISPR proteins before it is eliminated. Borges *et al.*[1] and Landsberger *et al.*[2] provide evidence that an initial unsuccessful phage infection that leads to expression of anti-CRISPR proteins generates an immunosuppressed bacterium that could then succumb to a subsequent phage infection, thereby facilitating successful phage replication.

infection limited this targeting of plasmid DNA, demonstrating that the bacterial cells were in an immunosuppressed state. Borges *et al.* demonstrated that phage-mediated immune suppression also occurs for another type of CRISPR–Cas system, called type II, suggesting that phage cooperation to enable immunosuppression of bacteria is a general principle that extends beyond a single type of CRISPR–Cas system.

The studies by Borges, Landsberger and their colleagues provide fresh insights into viral group dynamics, and join a growing body of evidence indicating that viruses can benefit from the group behaviour of a viral population. For example, phages can coordinate their infection dynamics using communication by small molecules to determine whether an individual phage will replicate in an infected cell or enter a dormant state termed lysogeny[7,8]. Perhaps phages also cooperate to overcome components of the bacterial immune system other than CRISPR–Cas, such as restriction enzymes or other antiviral defence systems[9].

Many questions remain to be answered concerning the bacterial immunosuppression generated by anti-CRISPR proteins. How long will a bacterium remain in this defenceless state? How does this state vary from cell to cell in a bacterial population? Although this cooperation strategy seems to provide one way for phages to neutralize CRISPR–Cas complexes, perhaps other strategies remain to be discovered that do not require the sacrifice of the first wave of infecting phages. ■

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