Bacterial dormancy curbs phage epidemics

One type of CRISPR–Cas bacterial defence system destroys phage and bacterial RNA, which leads to bacterial dormancy. Dormancy is found to limit viral spread, and also protects against unrelated viruses and viral mutants. See LETTER P.241

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An unseen war is being waged right under our noses, between microorganisms and their viral invaders. To fight the viruses called bacteriophages (phages) that target them, bacteria have evolved a diverse armoury of defences, which includes a range of protective immune systems called CRISPR–Cas.

On page 241, Meeske et al. reveal a curious twist in our understanding of the incredible variety of CRISPR–Cas defences, by demonstrating how a type of the CRISPR–Cas system that targets phage RNA protects bacteria from infection by DNA phages. The authors report that this CRISPR–Cas system responds to DNA phages by unleashing the destruction of both viral and bacterial RNA, which then causes infected bacterial cells to enter a state of dormancy that shuts down the cellular processes needed for viral replication. Meeske and colleagues reveal that this self-induced bacterial dormancy helps to suppress viral replication and viral outbreaks, including those caused by viral mutants that can escape other types of CRISPR–Cas defences, or viruses unrelated to the one that triggered dormancy. This defence response, in which the shutdown of an infected bacterial cell might benefit neighbouring bacteria, has interesting parallels with other types of defence system, such as bacterial abortive-infection systems or cell death in plants and animals that is induced by the innate branch of the immune system.

CRISPR–Cas systems are classified into six types termed I to VI. Most such systems capture and store short sequences of viral DNA as genetic ‘memories’ of phage invasion. These stored sequences are used to generate RNA guides that enable Cas enzymes to target and degrade viral DNA or RNA. Type VI systems are intriguing because they are the only ones that destroy viral RNA (Fig. 1) rather than DNA cry5, yet most phages have DNA rather than RNA genomes.

Type VI CRISPR–Cas systems, which use a Cas enzyme called Cas13, have previously been shown to respond to infection by RNA viruses by activating a form of indiscriminate (low sequence specificity) RNA-degrading activity called CRISPRi. In addition, when bacteria were engineered so that Cas13 targeted a messenger RNA encoded by a circular DNA sequence called a plasmid, bacterial growth was impaired. This suggested that, in the absence of phage infection, Cas13 activation, and its indiscriminate RNA destruction, led to bacterial-cell dormancy. But what role dormancy has, if any, in the antiviral defence processes remained an unanswered question.

To address this issue, Meeske et al. studied a type VI defence system, using the bacterium Listeria ivanovii and the DNA phage øRR4. The authors engineered the type VI system to guide Cas13 to target different øRR4 viral sequences and then analysed how effectively this system provided antiviral defence. Cas13 did indeed provide defence when targeted to viral mRNAs and, surprisingly, protection was achieved regardless of whether or not the targeted viral mRNAs corresponded to genes that are essential for viral replication, or whether the genes were expressed early or late during viral infection. There was also extensive bacterial RNA degradation in the infected cells, which caused infected bacteria to enter a dormant state in which the bacterial cells were alive but could not replicate.

This form of Cas13-induced dormancy has considerable parallels with another class of phage-defence system called...
abortive-infection mechanisms. If infected by viruses, bacteria harbouring abortive-infection systems enter a dormant state or trigger cell death, which provides a population-level antiviral defence.

Does Cas13-induced dormancy after viral infection offer the wider bacterial population a form of protection called herd immunity, in which resistant individuals help to slow the spread of infection to sensitive members of the population? Meeske and colleagues demonstrated that phage-infected bacteria possessing type VI immunity do indeed provide antiviral cross-protection to neighbouring cells of the same strain that lack any immunity against the infecting virus. The authors also observed that, by engineering Cas13-induced dormancy in uninfected cells, bacteria that suppressed viral epidemics were generated, probably because these dormant cells can act as sacrificial ‘decoy’ cells that viruses fail to infect successfully, which thereby depletes the viral population.

A study of type I-E CRISPR–Cas activity also reported bacterial-cell shutdown, but the effect was probably due to insufficient phage clearance, leading to a stalemate between bacterial defences and viral replication. By contrast, Meeske et al. demonstrate that the bacterial dormancy induced by Cas13 occurs through the active process of indiscriminate RNA degradation that is triggered following the recognition of viral RNA.

A challenge faced by CRISPR–Cas systems is if viral genetic mutants arise that escape recognition by the defence system. Meeske et al. showed that outbreaks of such mutant viruses are limited by the presence of wild-type viruses that cause Cas13–induced bacterial dormancy. Similarly, the authors found that when bacteria with type V1 defences against ϕRR4 were exposed to an unrelated virus, the level of infection by the unrelated virus was reduced if cells were also exposed to ϕRR4. This type of broad-spectrum defence might offer advantages over CRISPR–Cas systems that do not induce dormancy and in which infection by viral mutants is unaffected by the presence of wild-type viruses.

The scarcity of type VI defence in nature compared with other CRISPR–Cas systems argues that the type VI strategy might not always be superior. Indeed, other CRISPR–Cas systems have methods for dealing with mutant viruses, such as using feedback loops termed priming to update target memory or having enough flexibility to also recognize mutant forms of the viral target. Probably, each type of strategy that fights back against viral mutants has different costs and benefits in particular ecological settings.

The Cas13-induced dormancy response has interesting implications for how type VI systems form memories of viral infections. Because viral DNA or RNA is needed to form CRISPR–Cas memories, viral infection and subsequent bacterial-cell survival are necessary to update a type VI defence against viruses, including those not previously encountered, and mutated viruses. In type II systems, failed infections by defective viruses can allow a memory update without causing cell death. This might also offer a way for type VI systems to acquire new memories of viral infection. However, it would be more beneficial if some virus-infected cells clear the infection and recover from Cas13-induced dormancy. If dormancy helps bacteria to survive viral infection, it would be expected that the bacterial memories of DNA viruses stored by type VI systems would be biased towards targeting viral genes expressed early in the course of infection, thereby allowing the rapid suppression of viral infection and greater opportunity for bacterial recovery.

Intriguingly, the authors found that dormancy induced by targeting Cas13 to a bacterial mRNA could be reversed in the absence of viral infection. However, whether or not a bacterial cell can survive viral infection probably depends on how far infection has progressed before dormancy is initiated. Bacterial dormancy triggered by certain RNA-targeting abortive-infection systems, such as ToxIN, is reversible in the absence of viral infection, but it is unknown whether this is also the case for dormancy triggered by viral infection. Type III CRISPR–Cas systems activate enzymes that cause indiscriminate RNA destruction after the specific recognition of viral sequences, and this RNA destruction is inactivated when viral infection is suppressed. If cells can ‘wake’ from the dormancy induced by the type VI system, then perhaps a similar system of temporal control of nonspecific RNA degradation might be responsible. Alternatively, the activity of other bacterial factors or other antiviral defence systems might aid bacterial recovery after viral infection.

Meeske and colleagues’ findings reinforce the idea that CRISPR–Cas defence systems are context-dependent. Further studies will be required to better understand how these diverse defence systems in different bacteria tackle the various bacteriophages that they naturally encounter.

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