Virology

Killing the messenger to evade bacterial defences

Urs Jenal

Many mysteries remain about how antiviral responses shape the ability of viruses to infect bacteria. The finding that viruses interfere with signalling mediated by molecules called second messengers sheds light on bacterial defences. **See p.522**

In ancient times, the delivery of an unwelcome message by an envoy to those in high office posed a risk of retribution for the envoy. In Sophocles' play *Antigone*, a guard says, "No man delights in the bearer of bad news." Moreover, neutralizing information carriers is an effective manoeuvre for undermining an enemy's lines of communication during war. It turns out that viruses that infect bacteria (bacteriophages, or phages) optimize predation of their microbial hosts by targeting messengers needed for defence systems. Hobbs *et al.*[1] report on page 522 that phages use nuclease enzymes to degrade microbial molecules called nucleotide second messengers, and thereby subvert antiviral responses.

The discovery of nucleotide second messengers dates back to the observation that the hormone adrenaline does not enter human cells, but instead stimulates the formation of a distinct chemical substance (a second messenger) in the cell membrane. The nucleotide molecule cyclic AMP (cAMP) was found to act as an intermediate of hormone function[2], and another nucleotide, cyclic GMP (cGMP), was later also identified as a second messenger. The repertoire of known nucleotide second messengers grew[3–5] with the discovery that bacteria produce messengers using another type of nucleotide structure — a cyclic dinucleotide. Such molecules include c-di-GMP, c-di-AMP and cGAMP. Of these, c-di-GMP and c-di-AMP act as signals to control bacterial growth and behaviour, whereas cGAMP (specifically in a molecular configuration called 3′,3′-cyclic GMP–AMP) has a key role in defence against phages, by triggering bacterial cell death through a process called abortive infection[6].

Intriguingly, mammalian cells use a chemically modified version of cGAMP — in the 2′,3′-cyclic GMP–AMP (2′,3′-cGAMP) molecular configuration — in their own antiviral immune response. During infection, viral DNA is perceived in mammalian cells by a sensor protein called cGAS, which then produces 2′,3′-cGAMP as a messenger to activate the protein STING (refs 7, 8). STING binds to 2′,3′-cGAMP (and other cyclic dinucleotides) and initiates a signalling cascade that leads to the production of antiviral factors, such as the protein interferon[9,10].

These mammalian studies established the cGAS–STING pathway as central to a branch of defence called innate immunity[11]. The discovery that bacteria use cGAS-like proteins in their defence against phage infection indicates that mechanisms of antiviral response are evolutionarily conserved from bacteria to humans[12]. When bacteria sense infection by phages, various members of the family of cGAS-like proteins make a diverse range of cyclic di- and trinucleotides[13–15]. These second messengers, which function in a defence mechanism called the cyclic-oligonucleotide-based antiphage signalling system (CBASS), then trigger an abortive-infection response by interacting with downstream components, which directly and rapidly induce bacterial self-killing[6,16]. Other nucleotide second messengers, called cCMP and cUMP, have now joined the list of molecules involved in bacterial defence[17]. These systems are called the pyrimidine cyclase system for antiphage resistance (Pycsar) and, like their CBASS counterparts, they occur in a wide range of microorganisms.

Given the prominent role of nucleotide second messengers in bacterial defences, Hobbs and colleagues speculated that phages have evolved mechanisms to block such systems. They infected *Escherichia coli* and *Bacillus subtilis* bacteria with an array of phages, and assessed the stability of various cyclic nucleotides in microbial extracts. From this, they discovered activities leading to the degradation of CBASS and Pycsar messengers (Fig. 1). Investigating the enzymes responsible and the respective phage genes involved, the authors identified Acb1 as an anti-CBASS nucleotide-degrading enzyme (a nuclease) in bacteriophage T4, and Apyc1 as an anti-Pycsar nuclease for bacteriophage SBSphiJ.

Acb1 and Apyc1 can target a wide range of cyclic nucleotides. This discovery, and the observation that the genes encoding these nucleases are commonly found in phages, generally clustering in the viral genome

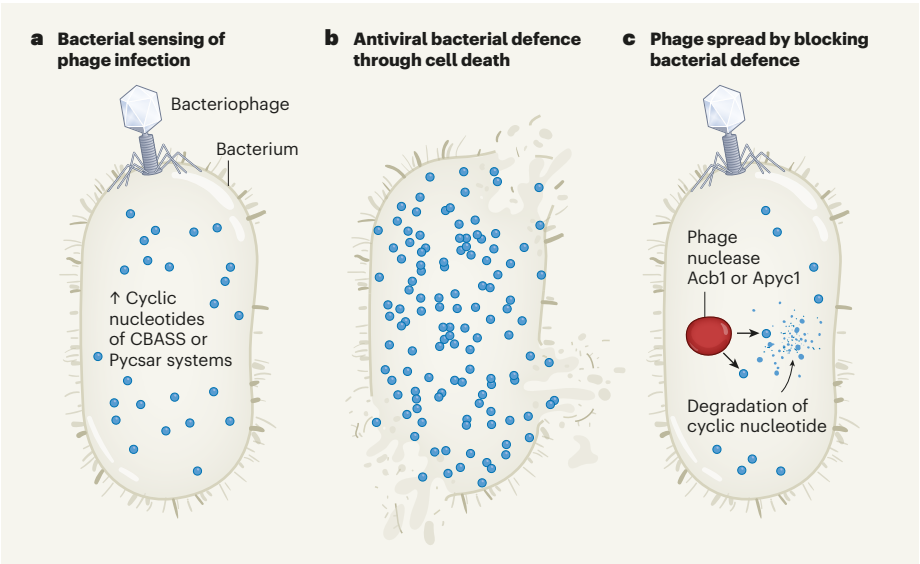

Figure 1 | Bacterial strategies to neutralize antiviral defences. Hobbs *et al.*[1] reveal a previously unknown mechanism that helps a virus (called a bacteriophage, or phage) to infect bacteria. **a**, Bacteria can launch antiviral responses through accumulation of bacterial molecules called cyclic nucleotides (various versions of these nucleotides are involved, such as cyclic mono-, di- or trinucleotides). Depending on the type of cyclic nucleotide, these defence responses are said to form either the cyclic oligonucleotide-based antiphage signalling system (CBASS) or the pyrimidine cyclase system for antiphage resistance (Pycsar). **b**, The defence response results in bacterial cell death, which prevents phage spread. **c**, The authors report that phages can block such defences using phage-encoded nuclease enzymes to degrade the cyclic nucleotides involved. Hobbs *et al.* identifed the nucleases Acb1 (anti-CBASS) and Apyc1 (anti-Pycsar) as functioning in this way. Bacterial cell death is not triggered by this degradation, enabling phage propagation.

alongside other genes involved in evasion of bacterial defences, argues that the nucleases have a key role in preventing bacteria from launching an abortive-infection defence. Hobbs *et al.* confirmed this by demonstrating that Acb1 and Apyc1 interfere with CBASS- and Pycsar-mediated immunity, and they present evidence that phage mutants lacking these nucleases have a greatly reduced ability to evade microbial defences mediated by cyclic nucleotides.

The authors observed that, although Acb1 is relatively nonspecific in terms of its nucleotide targeting, to be effectively cleaved, the target molecule must contain at least one nucleotide that contains the base adenine. It is unknown whether this is due to mechanistic constraints, is the result of phage nucleases having evolved to avoid being quenched by cyclic nucleotides they do not need to target, or is to circumvent an unfavourable physiological response by bacteria during infection. For instance, many bacteria use c-di-GMP to control and coordinate various aspects of cell growth and behaviour[18], and enzymes interfering with this complex regulatory network might therefore perturb optimal phage propagation. Consistent with this, c-di-GMP seems to be used as a defence signal only by specific groups of bacteria that do not exploit this molecule for their own cellular regulation[16]. Nevertheless, the interplay between host- and phage-mediated control of nucleotide messengers remains poorly understood. Hobbs *et al.* observed that structurally related versions of Apyc1 are encoded in bacterial genomes. This raises the possibility that these function to actively regulate Pycsar defences, or that other regulatory networks harnessing nucleotide second messengers await discovery.

Hobbs and colleagues' findings add to a growing list of viral components that interfere with cyclic nucleotide defence signals in the various kingdoms of life. This list includes poxins[19] — nucleases from mammalian viruses called poxviruses that obstruct the cGAS–STING pathway by degrading 2′,3′-cGAMP — as well as viral nucleases that degrade molecules called cyclic oligoadenylates, to interfere with defences (mediated by a mechanism known as CRISPR) in microorganisms called archaea[20].

One could speculate that the remarkable diversification of nucleotide second messengers in bacteria is crucial for maintaining antiphage defences in the face of constant evolutionary pressure by their phage predators. Indeed, no single type of phage investigated by Hobbs *et al.* could degrade all known cyclic nucleotides involved in bacterial defence, despite the broad substrate specificities of the phage nucleases. Given the growing list of bacterial defence mechanisms being discovered, and the possibility that these represent a limiting factor in the development of therapies that harness phages to combat bacterial infections, then engineering phages to have broader nuclease activities might be a suitable way to equip these viruses with maximal and far-reaching antimicrobial capacity.

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1. Hobbs, S. J. *et al. Nature* **605**, 522–526 (2022).
2. Sutherland, E. W. & Robison, G. A. *Pharmacol. Rev.* **18**, 145–161 (1966).
3. Ross, P. *et al. Nature* **325**, 279–281 (1987).
4. Witte, G., Hartung, S., Büttner, K. & Hopfner, K.-P. *Mol. Cell* **30**, 167–178 (2008).
5. Davies, B. W., Bogard, R. W., Young, T. S. & Mekalanos, J. J. *Cell* **149**, 358–370 (2012).
6. Cohen, D. *et al. Nature* **574**, 691–695 (2019).
7. Sun, L., Wu, J., Du, F., Chen, X. & Chen, Z. J. *Science* **339**, 786–791 (2013).
8. Ablasser, A. *et al. Nature* **498**, 380–384 (2013).
9. Wu, J. *et al. Science* **339**, 826–830 (2013).
10. Burdette, D. L. *et al. Nature* **478**, 515–518 (2011).
11. Hopfner, K.-P. & Hornung, V. *Nature Rev. Mol. Cell Biol.* **21**, 501–521 (2020).
12. Wein, T. & Sorek, R. *Nature Rev. Immunol.* https://doi.org/10.1038/s41577-022-00705-4 (2022).
13. Whiteley, A. T. *et al. Nature* **567**, 194–199 (2019).
14. Ye, Q. *et al. Mol. Cell* **77**, 709–722 (2020).
15. Govande, A. A., Duncan-Lowey, B., Eaglesham, J. B., Whiteley, A. T. & Kranzusch, P. J. *Cell Rep.* **35**, 109206 (2021).
16. Morehouse, B. R. *et al. Nature* **586**, 429–433 (2020).
17. Tal, N. *et al. Cell* **184**, 5728–5739 (2021).
18. Jenal, U., Reinders, A. & Lori, C. *Nature Rev. Microbiol.* **15**, 271–284 (2017).
19. Eaglesham, J. B., Pan, Y., Kupper, T. S. & Kranzusch, P. J. *Nature* **566**, 259–263 (2019).
20. Athukoralage, J. S. *et al. Nature* **577**, 572–575 (2020).

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Developmental biology

Tension around the clock

Miki Ebisuya & Xavier Trepat

The formation of body segments in vertebrate embryos has long been attributed to the spatio-temporal patterning of molecular signals. But segment length in zebrafish is now found to be adjusted by tissue mechanics. **See p.516**

Not all clocks are precise. The segmentation clock is a molecular oscillator that regulates the timing of the formation of somites — multicellular blocks that give rise to bilateral structures such as ribs and skeletal muscle. During vertebrate development, somites periodically bud off on both sides of an embryonic structure called the neural tube[1], with one pair of somites being formed during each cycle of the segmentation clock. The somites' initial volume is determined by both the frequency of the clock's oscillation and the speed of cell movement[2]. But Naganathan and colleagues[3] reveal on page 516 that the initial length of somites is surprisingly imprecise. The authors uncover a mechanism by which length is adjusted during somite formation in zebrafish. Rather than being based on the segmentation clock, this mechanism hinges on a single mechanical property of the somite — its surface tension.

"These findings imply a contribution of tissue mechanics to the symmetrical appearance of somites."

Using sophisticated 3D imaging of zebrafish embryos, Naganathan *et al.* first observed that the head-to-tail (antero-posterior) length of newly formed somites was highly variable. However, over the course of two hours, the somites adjusted to a target length of 51 micrometres.

The authors considered several potential mechanisms to explain this length adjustment. First, they tested whether it could be understood through the effects of the segmentation clock. They ruled out this possibility by showing that perturbing the clock did not change the dynamics of length adjustment. Second, they considered mechanisms based on crosstalk between left and right somites. Again, they ruled this out, showing that disrupting somite formation on just one side of the embryo did not affect length adjustment on the other side. A third possibility was that the length adjustment could be explained by differential overall growth rates in somites. But this possibility, too, was rejected when the authors showed that somite volume remained constant during length adjustment. Instead, they found that changes in the antero-posterior length of the somites were balanced by changes in the centre-to-side (mediolateral) length.

Naganathan and co-workers then proposed that the adjustment of somite length is driven by a mechanical property of the somite, namely,