Viruses wrap up bacterial defence systems

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SUBJECT STRAPLINE

Microbiology

STANDFIRST

Bacteria use diverse defences against their viral predators, called bacteriophages. Two new studies highlight methods for identifying counter-defences in viral genomes and reveal striking modes of defence inhibition. See p.XXX and p.XXX.

MAIN ARTICLE

Bacteria use a diverse and broad set of defence systems to protect from infection by viruses called bacteriophages[1]. In turn, bacteriophages have evolved specialised counter-defence systems that ensure successful viral replication[2]. On page XXX of this issue., Yirmiya *et al*.[3], rationally identify and characterise conserved counter-defence gene families targeting three distinct bacterial defences. They go on to show that Tad2 proteins are molecular sponges sequestering immune signals that would otherwise activate Thoeris defences and stop viral replication. On page XXX., Antine *et al*.[4], demonstrate how defence system Gabija is sequestered and inhibited by an octamer of counter-

defence Gad1 wrapping around the entire Gabija complex. These studies highlight an effective method for the identification of counter-defences and provide key insights into their mechanisms of inhibition. Together they expand and deepen our understanding of the genomic organisation and evolutionary diversity of bacteriophage counter-defences.

Yirmiya *et al*.,[3] gathered genetically similar bacteriophages and assayed their ability to grow on bacterial hosts expressing a range of previously identified defence systems[1]. Quantitative assessment of replication allowed each bacteriophage to be categorised as sensitive or resistant to the target defence system. The authors identified bacteriophages with potential counter-defence activity against five defence systems; Thoeris, Hachiman, Gabija, Septu and Lamassu[1]. Comparative genomics allowed the authors to then identify candidate counter-defence genes encoded within the genomes of resistant bacteriophages that were not present in sensitive bacteriophages against three defences (Thoeris, Hachiman and Gabija) (**Fig. 1a**).

To verify whether these genes do counter bacterial defences, Yirmiya *et al*.[3] generated genetically modified bacteriophages wherein the counter-defence gene was either deleted from the genomes of resistant phages, or inserted into the genomes of sensitive phages. Testing these modified bacteriophages against bacteria expressing the target defence system confirmed counter-defence activity. Subsequent phylogenetic searches mapped the distribution of counter-defence genes in bacteriophages and prophages (bacteriophage genomes integrated in the bacterial genome). Similar to the clustering of defence systems within "islands" on bacterial genomes, counter-defences appear to cluster in bacteriophage genomes, an observation that was also made in previous studies[5–7], suggesting future "guilt-by-association approaches" will identify many more candidates. Interestingly, prophages encoding counter-defence genes often associated with hosts encoding the corresponding defence system, allowing the prophage to survive in the host whilst the niche is protected from other bacteriophage species.

Thoeris protein ThsB detects bacteriophage infection and generates a nucleotide-derived signalling molecule, 1''-3' gcADPR, which in turn activates ThsA and induces depletion of cellular NAD^+, preventing phage replication[8]. A previously identified Thoeris counter-defence protein, Tad1 (Thoeris anti-defence 1), acts as a molecular sponge, by binding 1''-3' gcADPR and thereby preventing ThsA activation[8]. Yirmiya *et al*.[3], identified a new candidate counter-defence against Thoeris, named Tad2, and demonstrated through genetic, biochemical and structural analyses, that Tad2 also sequesters 1''-3' gcADPR, forming a tetrameric assembly that bound the ligand in a conformation similar to Tad1. Despite these mechanisitic similarities however, Tad2 appears to be evolutionary unrelated to Tad1, being genetically and architecturally highly distinct from Tad1. Together with previous studies demonstrating the 'molecular sponge' as a counter-defence strategy against other bacterial defences[8,9], this suggests that molecular 'sponging' of immune signaling molecules may have evolved multiple times during the longstanding evolutionary battle between bacteria and their viruses. Yirmiya *et al*.,[3] also identified and solved the structure of Had1, targeting Hachiman. Using Had1 as a reagent

to block Hachiman might in the future provide greater insight into the currently unknown Hachiman mechanism of action. The team also found a third counter-defence protein called Gad1, which targets Gabija.

The article from Antine *et al*.[4] outlines biochemical and structural characterisation of both *apo* (unbound) and Gad1-bound Gabija complexes. Gabija encodes two proteins, GajA, which forms a tetrameric OLD nuclease/TOPRIM core that binds two dimers of a helicase, GajB. In cells, both components are required to cleave bacteriophage DNA based on recognition of specific sequences (**Fig. 1b, *left***)[10]. Gad1 is unusual as it is significantly larger (35 kDa) than the majority of counter-defence proteins identified so far. Cryo-EM analysis of the Gad1-bound GajAB complex showed remarkable oligomerisation of Gad1, wherein the highly extended and flexible protomers form an octamer that encircles the entire GajAB, wrapping it up tight (**Fig. 1b, *right***). In effect, GajAB becomes sequestered and when tested biochemically, Gad1 prevents DNA binding and cleavage, potentially due to shielding of DNA-binding sites on the surface of GajA.

Counter-defence systems have been identified previously, targeting restriction-modification, CRISPR-cas, CBASS, ToxIN and many other defence systems[2]. Their modalities range from direct binding of defence effectors, mimicry of nucleic acid substrates, sequestration or degradation of signalling molecules, and many more. The use of guilt-by-association analysis to identify putative defence systems clustered in "defence islands" has led to a recent flurry in the identification and characterisation of new defence systems and activities. In a similar vein, the current studies use comparative genomics for the discovery of counter-defence genes, by leveraging the systematic organisation of "counter-defence islands". This will no doubt add to an equally vast expansion of newly identified counter-defences.

The evolved products of the interplay between bacteria and bacteriophages underpin modern biotechnology, having led on cloning and now genome editing. Expanding our knowledge of these systems can only increase the number of research tools that are available, which may yet become important tools for tackling the encroaching problems of food security, an aging population, and antimicrobial resistance. On this final example, bacteriophages are a proven alternative to antibiotics for the treatment of bacterial infections. The success of bacteriophage therapy relies upon understanding host-virus interactions, and as demonstrated by these studies, personal medicine might target specific recalcitrant pathogens by engineering bacteriophages to overcome host defences.

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COMPETING INTERESTS

The authors declare no competing interests.

REFERENCES

1. Doron, S. *et al.* Systematic discovery of antiphage defense systems in the microbial pangenome. *Science* **359**, eaar4120 (2018).

2. Samson, J. E., Magadan, A. H., Sabri, M. & Moineau, S. Revenge of the phages: defeating bacterial defences. *Nat Rev Microbiol* **11**, 675–687 (2013).

3. Yirmiya, E. *et al.* Phages overcome bacterial immunity via diverse anti-defense proteins. *bioRxiv* 2023.05.01.538930 (2023) doi:10.1101/2023.05.01.538930.

4. Antine, S. P. *et al.* Structural basis of Gabija anti-phage defense and viral immune evasion. *bioRxiv* 2023.05.01.538945 (2023) doi:10.1101/2023.05.01.538945.

5. Pawluk, A. *et al.* Inactivation of CRISPR-Cas systems by anti-CRISPR proteins in diverse bacterial species. *Nat Microbiol* **1**, 16085 (2016).

6. Pinilla-Redondo, R. *et al.* Discovery of multiple anti-CRISPRs highlights anti-defense gene clustering in mobile genetic elements. *Nat Commun* **11**, 5652 (2020).

7. Rousset, F. *et al.* Phages and their satellites encode hotspots of antiviral systems. *Cell Host Microbe* **30**, 740-753.e5 (2022).

8. Leavitt, A. *et al.* Viruses inhibit TIR gcADPR signalling to overcome bacterial defence. *Nature* **611**, 326–331 (2022).

9. Huiting, E. *et al.* Bacteriophages inhibit and evade cGAS-like immune function in bacteria. *Cell* **186**, 864-876.e21 (2023).

10. Cheng, R. *et al.* A nucleotide-sensing endonuclease from the Gabija bacterial defense system. *Nucleic Acids Res* **49**, 5216–5229 (2021).

FIGURE LEGEND

Figure 1. Identification of new bacteriophage counter-defence proteins that display diverse modes of action. Bacteria use a broad range of defence systems to protect from viruses called bacteriophages. Bacteriophages have evolved counter-defence genes to counter the host immunity. **a**, Genetically similar bacteriophages are tested against individual defence systems and sorted into those bacteriophages that are sensitive and thereby prevented from replicating by the defence system, and those that are resistant. Comparative genomics between the two groups allows identification of candidate genes for putative counter-defence proteins. Genetically modifying bacteriophages to either remove or add candidate counter-defence genes will then confirm function. **b**, Bacteriophages that are sensitive to Gabija defence systems have their DNA degraded by the GajA OLD nuclease, as part of the GajAB complex. Bacteriophages expressing counter-defence protein Gad1 wrap up the GajAB complex in an octamer of Gad1 proteins. Complex sequestration and steric occlusion of DNA-binding sites by Gad1 prevents GajAB activity, ensuring immune evasion and successful bacteriophage replication.