Microbiology

Bacterial twist to an antiviral defence

Karen L. Maxwell

The discovery of an antiviral defence system in bacteria that shares some components with a key antiviral defence pathway in animals provides insight into how this important response might have evolved. **See p.691**

Humans face a daily threat of infection by harmful viruses. To repel them, our immune system mounts an immediate response following invasion that depends on its ability to recognize general characteristics indicating that viruses are foreign entities. This type of reaction, generated by an ancient branch of the immune system known as innate immunity, occurs in all plants and animals. Many genes involved in innate immune responses are evolutionarily conserved and encode proteins that are used for defence purposes in different species[1–3]. Cohen *et al.*[4] report on page 691 that some bacterial species fight viral infections by using an innate immune mechanism that is related to one of the central components of innate immunity in animals called the cGAS–STING pathway. Their findings reveal that this crucial antiviral defence system in animals might have its evolutionary roots in bacteria.

There has been a rise in evidence indicating that the defence systems mediating innate immunity in animals have counterparts in bacteria. For example, protein components called TIR domains, which are present in defence proteins in mammals and plants, can recognize molecular hallmarks of disease-causing agents known as PAMPs, and then trigger an immune response. TIR domains are evolutionarily conserved in bacteria, protecting them from viruses called phages[5]. Another such example is the antiviral machinery that targets RNA and depends on proteins called Argonautes, found in plants and animals. This system also has a role in defence responses in bacteria and the single-celled organisms known as archaea[6,7].

The evolutionary conservation of these innate immune mechanisms in bacteria and mammals suggests that such pathways might have first arisen in bacteria as protection against phages, and have since evolved into different, but related, defences across the tree of life. One key open question is how many of the innate immune defences found in animals might have evolved from ancient bacterial systems.

When the cGAS–STING pathway[8,9] in animals detects invading viruses in a cell, it activates a response that either mediates antiviral defences or triggers cell death[10]. The cGAS enzyme functions in this defence by sensing and binding to double-stranded viral DNA, and then inducing[11] the production of a type of signalling molecule called cGAMP, which is termed a cyclic dinucleotide. The binding of cGAMP to the STING protein sets off a signalling cascade that unleashes an antiviral response.

Cohen and colleagues analysed regions of bacterial genomes in which defence genes are clustered, and noticed that the gene encoding cGAS was often located near genes whose products involved in other antiphage defence systems, such as CRISPR–Cas. The authors therefore wondered whether cGAS might have a role in antiphage defences.

To test this idea, Cohen *et al.* engineered bacteria lacking a cGAS system to express genes encoding such systems. The authors tested two representative cGAS systems (comprising the gene encoding cGAS and three adjacent genes) from the bacterial species *Vibrio cholerae* and *Escherichia coli*. Both cGAS systems conferred a resistance to infection by diverse phages. When the authors disrupted the DNA sequence of the cGAS-system genes, resistance to phage infection was completely lost – confirming that bacteria use this cGAS signalling pathway for antiviral defence, much as do eukaryotes (multicellular organisms that have a nucleus in their cells). The authors called this antiphage defence system cyclic-oligonucleotide-based anti-phage signalling system (CBASS). Genes encoding cGAS proteins are present in approximately 10% of all sequenced bacterial genomes[12], suggesting that CBASS systems have a widespread role in antiphage defences.

The pathway downstream of cGAMP production in bacteria differs from that in animals. The authors report that, in bacteria, cGAMP production activated a phospholipase enzyme in some CBASS systems (Fig. 1). This activated phospholipase then degraded phospholipid molecules in the bacterial cell membrane, killing the infected bacterium. Such cellular 'suicide' could protect a bacterial cell population because the destruction of infected cells through this process prevents the phage from spreading to neighbouring bacteria.

In some bacterial species, the CBASS defence systems identified by Cohen and colleagues lacked a phospholipase component. These systems instead encoded proteins that might trigger cell suicide through alternative

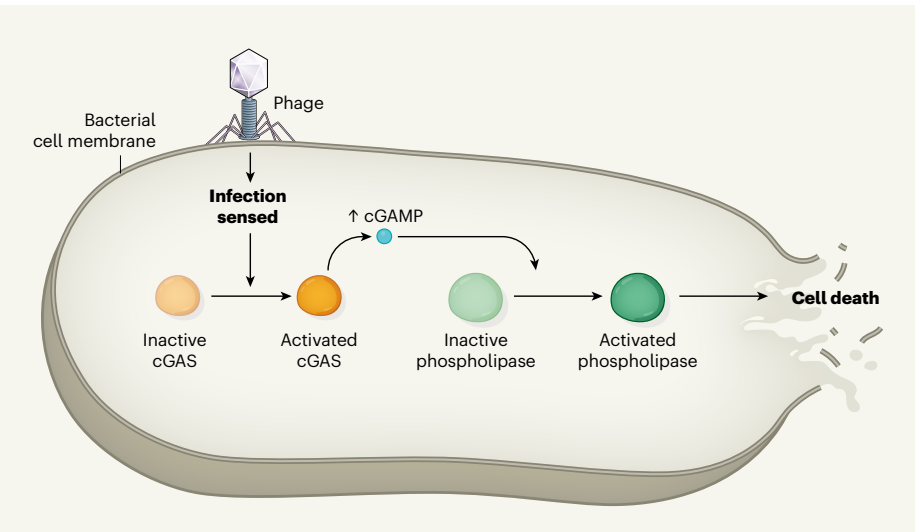

Figure 1 | A bacterial defence against viral infection. Cohen *et al.*[4] report a defence system that is used by bacteria to fight infection by bacterium-infecting viruses called phages. After phage infection is sensed (through an unknown mechanism), the enzyme cGAS is activated, producing the molecule cGAMP. Such changes in cGAS activity and cGAMP levels in response to viral infection also occur in a range of multicellular organisms, including humans. In the bacterial system, the rise in cGAMP can lead to activation of a phospholipase enzyme that degrades phospholipid molecules in the bacterial cell membrane. This process kills the bacterial cell and can stop viral infection from spreading to neighbouring cells.

mechanisms, such as degrading the bacterial genome or creating a hole in the cell membrane though the action of a pore-forming protein. But whether these systems kill cells in such ways remains to be tested. In some cases, the CBASS systems encoded a protein in which a TIR domain was fused to a STING domain similar to that in eukaryotes. The evolutionary conservation of these domains in an antiviral defence system in bacteria suggests that they might represent the ancient evolutionary origin of the eukaryotic cGAS–STING defence system.

Although some CBASS systems had only cGAS genes and components required for bacterial cell death, others had genes whose products were associated with ubiquitination, a protein-modification pathway in eukaryotic cells. In this process, a protein called ubiquitin is attached to a target by an enzyme-mediated reaction. CBASS systems included proteins that have several components associated with eukaryotic ubiquitination: E1 and E2 domains, typically found in enzymes that mediate ubiquitin activation and transfer, respectively, and JAB domains, which are found in proteins that remove ubiquitin from targets. Ubiquitination fine-tunes the length and intensity of innate immune responses in animals[13]. This provides yet another link connecting bacterial and animal antiviral responses. The ubiquitination components of the *E. coli* CBASS system were required for defence against some but not all phages, suggesting that these proteins might allow systems to recognize specific phage proteins or features, rather than being a more general property of phages – thereby refining the activity of these systems.

Antiphage defence systems in bacteria can be a target of phage-encoded inhibitor proteins. For example, phage proteins can block CRISPR–Cas defences[14]. It is highly probable that some phages have evolved ways to inhibit CBASS systems. Different CBASS systems encode a diverse set of cyclic-oligonucleotide signalling molecules and components, suggesting that cell suicide occurs through a number of mechanisms. The diversity of these CBASS-system components is probably driven by the need to evade a phage counter-attack if, for example, a phage-encoded protein could inactivate a particular cyclic-oligonucleotide signalling molecule. The selective pressure from antiphage systems that phages encounter would inevitably lead to the evolution of countermeasures in these viruses. An exciting area for future research will be to search for such phage inhibitors of CBASS systems.

One key aspect of cGAS function in bacterial defence that remains unknown is which signal the immune system detects to recognize that a viral infection is occurring. In eukaryotes, any viral double-stranded DNA in the cytoplasm can be recognized as a foreign entity because eukaryotic DNA is usually confined to the nucleus and absent from the cytoplasm. To distinguish cytoplasmic viral DNA from bacterial DNA, a bacterium lacking a nucleus would presumably require a sensor with a nuanced capacity to identify foreign DNA. One possibility is that CBASS systems recognize phage DNA specifically in the linear, relaxed state that occurs immediately after it has entered the bacterial cell. Perhaps the proteins that have E1, E2 and JAB domains in CBASS systems provide further refinement to aid the success of this aspect of phage recognition.

Cohen and colleagues' study is particularly remarkable for highlighting the striking parallels between innate immunity in eukaryotes and bacteria. The number of known bacterial antiphage systems is growing rapidly[5,15,16], and it is probable that many more such exciting connections remain to be uncovered.

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Cancer

Teamwork by T cells boosts immunotherapy

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Immunotherapy treatment harnesses CD8 T cells of the immune system to kill tumour cells. The finding that CD4 helper T cells contribute to the success of this treatment in mice might offer a way to improve clinical outcomes. **See p.696**

Immune cells called CD8 (or cytotoxic) T cells can target and kill cancer cells, and immunotherapies that boost this process are in clinical use. However, for reasons that are not fully clear, it is hard to predict whether a person will respond to this treatment. On page 696, Alspach *et al.*[1] report mouse studies revealing that another type of immune cell, called a CD4 cell (also known as a helper T cell), has a crucial role in aiding CD8 T cells to target tumours after immunotherapy.

Mutations in tumour cells can give rise to abnormal proteins, fragments of which – termed neoantigens – are displayed on the surface of cells bound to major histocompatibility complex (MHC) molecules. If a neoantigen is recognized by a CD8 T cell, this cell can target and kill any tumour cells that express the neoantigen. However, this cytotoxic response can be blocked, for example by an immunosuppressive environment surrounding a tumour. Immunotherapy treatments called immune-checkpoint blockade or immune-checkpoint therapy can counteract such problems to enable CD8 T cells to unleash an effective immune response against the tumour.

Much immunotherapy research focuses on CD8 T cells. However, there is emerging evidence that CD4 T cells might have a key role in tumour-targeting immune responses[2,3].

Alspach and colleagues sought to identify the minimal immune-stimulating neoantigen requirement to drive an effective immune response in mice that were given an immunotherapy treatment. The authors studied mice that had a type of tumour to which the immune system does not normally respond, and they engineered such tumours to express neoantigens. The neoantigen termed mLAMA4 is recognized by CD8 T cells[4], and the neoantigen termed mITGB1, recognized by CD4 T cells, was identified by the authors using a computational prediction method. In the absence of immunotherapy, the expression of these two neoantigens, either alone or together in a tumour, was insufficient to trigger an effective immune response against the tumour. However, if both neoantigens were expressed in animals receiving immunotherapy, the tumour regressed.

To check whether this response was simply