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MICROBIOLOGY

A stockpile of antiviral defences

The full list of weapons used by bacteria against viruses is not known. A computational approach has uncovered nine previously unidentified antiviral systems, encoded by genes near known defence genes in bacterial genomes.

SÉBASTIEN LEVESQUE & SYLVAIN MOINEAU

Fighting viruses is no easy task. Bacteria have survived attacks by viruses called phages by evolving sophisticated defence strategies that enable them to thrive even in virus-rich ecosystems. However, phages have evolved counter-tactics to thwart such mechanisms[1], leading to a biological arms race. Now Doron *et al.*[2] report the identification of previously unknown antiviral systems in bacteria.

Anti-phage systems usually target key steps in viral replication. For example, some systems prevent phage binding to bacterial cells, whereas others block entry of the viral genome into the cell[3]. Certain bacterial proteins can halt intracellular phage replication[3,4]. Although this often leads to the death of infected cells, it can protect neighbouring cells from infection. Perhaps the best known anti-phage systems are restriction enzymes and CRISPR–Cas. These two systems[5–7], can cleave non-host DNA in a sequence-specific manner, and have also been widely adapted as molecular tools in the biological sciences.

As knowledge of the diversity of Earth's viruses has grown[8], along with the potential of using such information to develop further biotechnology tools, investigation into anti-phage systems has surged. Many lines of evidence have indicated that the list

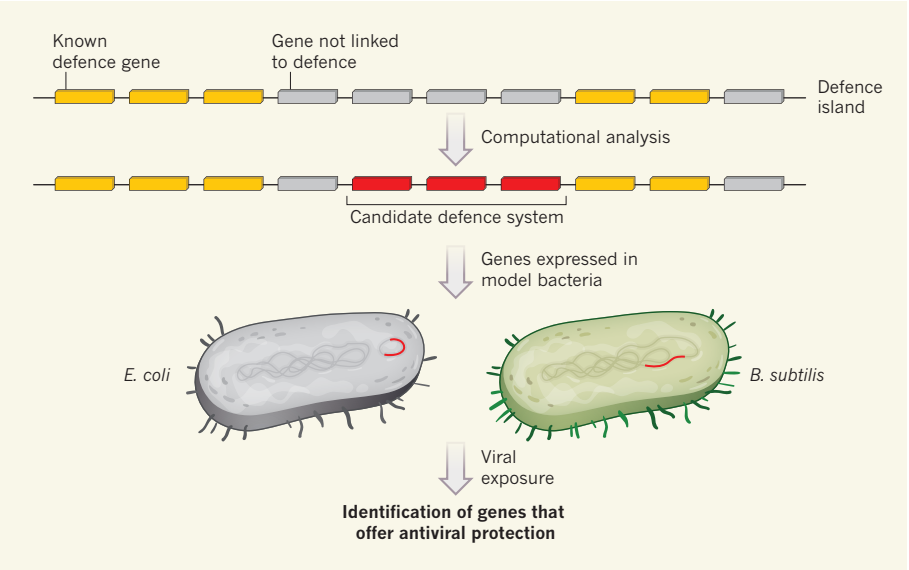

Figure 1 | Identifying antiviral systems in bacteria. Bacterial defence genes (yellow) are found in regions of the genome known as defence islands. Doron *et al.*[2] sought to identify more such genes by analysing genes within these islands that had not previously been linked to defence functions (grey). They used computational analysis involving a range of criteria, including whether the genes were located in defence islands in many different types of bacterium. The authors also identified neighbouring genes that might function together as a defence system. These proposed defence-system genes were then expressed in the model bacteria *Escherichia coli* and *Bacillus subtilis*. The bacteria were exposed to various viruses to test whether the genes offered protection against infection. The authors confirmed that nine of the defence systems they tested had antiviral functions.

of microbial-defence 'weapons' is probably far from complete.

Previous computational analyses have shown that defence genes cluster together in bacterial genomes in specific regions called defence islands[9]. Enter Doron *et al.*, armed with the knowledge[9] that these regions also contain many gene families that have unknown functions. The authors analysed more than 45,000 microbial genomes to find genes that are frequently found in defence islands. For their analysis, they grouped the encoded proteins into families that share a specific structural domain. Doron and colleagues analysed 14,083 protein families, and focused on those in which at least 65% of the encoding genes were located near known defence systems. These genes were then used as 'anchors' from which to investigate neighbouring genes, because defence genes are often found to be part of a series of consecutive genes that function together in the same defence process.

The authors pinpointed 335 families of interest. After further studies to identify gene clusters that are evolutionarily conserved across multiple genomes and in a broad distribution of microbes, they selected 28 such clusters for functional testing. They expressed the genes in two model bacteria: *Bacillus subtilis* and *Escherichia coli* (Fig. 1). In *B. subtilis,* the selected genes were integrated into the genome, whereas in *E. coli,* they were engineered into circular-plasmid DNA.

The bacteria successfully expressed at least one example of 26 of these candidate defence systems, as confirmed by RNA sequencing. They also expressed six known defence systems as controls. The bacteria were then exposed to a range of phages belonging to four distinct phage families known to infect them. Remarkably, nine of the 26 systems offered protection against at least one phage. These defence systems contained up to five genes. One system was present in 3% of the bacterial genomes analysed, and another was found in 4% of microbes investigated. The authors named the systems after mythological protective deities.

Some selected candidates had no anti-phage activity. This was not surprising, because they were tested under specific laboratory conditions and were expressed in hosts that do not normally express these genes: defence mechanisms are often effective only against specific phage groups. Indeed, only three of the six known defence systems used as controls provided protection against phages in the experiments. The authors speculated that some of the defence systems they had identified might specifically defend against plasmid introduction. In an experiment testing the efficiency of plasmid introduction into *B. subtilis*, they found that the presence of one of the defence systems substantially reduced the level of plasmid introduction. Altogether, the authors identified ten defence systems (nine antiviral and one antiplasmid) in various microbes.

Doron and colleagues proposed distinct modes of action for some of these defence mechanisms on the basis of the presence of specific domains in some of the bacterial proteins. For example, one protein has a TIR domain. This domain is a key component of the innate immune system of mammals, plants and invertebrates and it functions in signalling pathways activated in response to the recognition of infectious agents. However, in-depth mechanistic studies are needed to draw any conclusions about how these newly identified defence systems might function.

"Defence genes cluster together in bacterial genomes in specific regions called defence islands."

The discovery of this hidden stockpile of anti-phage weapons is exciting, and emphasizes the fact that the complete array of bacterial defence systems remains unknown. Doron and colleagues' experiments might even have missed some systems because of the technical methods they used. For example, some groups of genes tested might have been incompatible with the model bacteria used, or might provide protection only against phages that weren't tested. Indeed, the recent discovery of a major lineage of marine viruses[10] is a reminder that our inventory of viruses continues to expand.

The authors have convincingly demonstrated an effective computational approach for discovering bacterial defence systems. The presence of multiple such mechanisms in a given bacterium gives the microbe a robust safeguard against viral infection[11], so the decision to investigate defence islands was an astute one. In the never-ending battle between phages and bacteria, it will also be interesting to learn how phages have evolved to neutralize or circumvent these newly unmasked weapons. Rest assured, phages are here to stay, and are bound to mount a counter-attack. ■

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ASTROPHYSICS

Bounteous black holes at the Galactic Centre

X-ray observations have revealed a dozen stellar-mass black holes at the centre of the Galaxy, implying that there are thousands more to be found. The discovery confirms a fundamental prediction of stellar dynamics.

MARK R. MORRIS

A dense cluster of stars surrounds the supermassive black hole that lies at the Galactic Centre. Stars that live and die in the cluster are almost always held captive by the irresistible gravity of this strong concentration of mass. Consequently, the black-hole remnants left behind by the deaths of massive stars are predicted to have piled up in the central parsec (3.26 light years) of the Galaxy during its lifetime. Theoretical estimates of the number of stellar-mass black holes in this region range from the thousands to the tens of thousands[1–3]. Writing in a previous issue of *Nature*, Hailey *et al.*[4] reported on what could be the first observational evidence for such a black-hole cluster.

All stars emit X-rays, but only the brightest stellar X-ray sources at the centre of the Galaxy can be observed. Nevertheless, with a single field of view pointing towards the Galactic Centre, the Advanced CCD Imaging Spectrometer (ACIS) of NASA's space-based Chandra X-Ray Observatory has detected thousands of these sources. Almost all are found in close binary systems that comprise a normal star and a compact companion. The X-rays are generated by gas that is subjected to strong heating when it is pulled out of the normal star and transferred (accreted) onto, or into, its companion.