BACTERIAL PHYSIOLOGY

Putting the 'BREX' on phage replication

Bacteria have evolved multiple strategies to prevent phage infection, such as abortive infection, and the restriction–modification (R–M) and the CRISPR–Cas systems. Now, Goldfarb, Sberro, *et al.* describe a novel defence system, named bacteriophage exclusion (BREX), that is widespread in bacterial genomes and protects against lytic and lysogenic phage infection.

Bacterial genes that are involved in resistance against phages are commonly clustered in specific genomic locations, forming genomic defence islands. One such gene is *pglZ*, which encodes a phosphatase that has previously been implicated in a unique phage resistance phenotype in *Streptomyces coelicolor* called phage growth limitation (Pgl). Although the molecular mechanism of Pgl-mediated resistance is unknown, *S. coelicolor* strains carrying the Pgl system are susceptible to the first cycle of infection by the ΦC31 phage but are resistant to the progeny phages that are produced during this first cycle of infection.

To investigate the distribution of *pglZ*, Goldfarb, Sberro, *et al.* analysed more than 1,500 bacterial and archaeal genomes and detected genes homologous to *pglZ* in approximately 10% of the genomes, with a wide phylogenetic distribution. In most cases, *pglZ* is part of a six-gene cassette that the authors termed BREX; in addition to *pglZ*, it includes *pglX*, which encodes a methyltransferase that has also been associated with the Pgl phenotype, and *brxABCL*, which encodes an RNA-binding

anti-termination protein (BrxA), a protein of unknown function (BrxB), an ATP-binding protein (BrxC) and a protease (BrxL).

To test whether BREX is involved in the defence against phages, the authors cloned the complete BREX system from *Bacillus cereus* into *Bacillus subtilis*, which lacks an endogenous BREX system. Notably, BREX expression in *B. subtilis* strains conferred resistance against infection with both lytic and lysogenic phages.

So, what is the mechanism of BREX-mediated protection? Owing to the homology between some of the genes present in the BREX system and those that have been implicated in the Pgl phenotype, the authors assessed whether BREX also confers protection by limiting phage growth. Importantly, the authors could not detect Φ3T phage production following infection of BREX-expressing bacteria, which indicates that these cells are not susceptible to the first infection cycle and that BREX and Pgl provide resistance against invading phages through different mechanisms. Notably, they also showed that BREX does not prevent phage adsorption and is not an abortive infection system; however, similarly to R–M systems, BREX blocks phage DNA replication in infected cells. R–M systems rely on restriction enzymes that degrade foreign unmethylated DNA, whereas methylation of 'self' DNA prevents targeting of the bacterial genome. As the BREX cassette contains the PglX methyltransferase, the authors compared the methylation patterns

of DNA extracted from *B. subtilis* cells that express or lack BREX. Using single molecule, real-time (SMRT) sequencing, they detected methylation on the fifth position of a non-palindromic hexameric sequence (TAGGAG) in genomes of nearly all cells that expressed BREX, whereas cells lacking BREX did not carry this modification. However, unlike the conventional R–M systems, phage DNA was not cleaved or degraded in BREX-expressing cells. These findings led the authors to propose that the BREX system is a novel defence strategy that involves DNA methylation of the host cell and inhibits phage DNA replication.

Although additional studies are required to elucidate the molecular mechanism of BREX-mediated resistance, this study establishes BREX as a new defence system that is widespread among bacteria and that protects them from both lytic and lysogenic phage infection. Finally, given the advances in molecular biology that have resulted from the study of other bacterial defence systems, such as restriction enzymes and CRISPR–Cas, it will be interesting to follow the development of BREX as another potential tool for genetic manipulation.

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