Bacteriophage exclusion, a new defense system

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The ability to withstand viral predation is critical for survival of most microbes. Accordingly, a plethora of phage resistance systems has been identified in bacterial genomes (Labrie *et al*, 2010), including restriction-modification systems (R-M) (Tock & Dryden, 2005), abortive infection (Abi) (Chopin *et al*, 2005), Argonaute-based interference (Swarts *et al*, 2014), as well as clustered regularly interspaced short palindromic repeats (CRISPR) and associated protein (Cas) adaptive immune system (CRISPR-Cas) (Barrangou & Marraffini, 2014; Van der Oost *et al*, 2014). Predictably, the dark matter of bacterial genomes contains a wealth of genetic gold. A study published in this issue of *The EMBO Journal* by Goldfarb *et al* (2015) unveils bacteriophage exclusion (BREX) as a novel, widespread bacteriophage resistance system that provides innate immunity against virulent and temperate phage in bacteria.

See also: T Goldfarb *et al* (January 2015)

It is well documented that viruses engage in a continuous co-evolutionary arms race with their microbial hosts, which has generated a broad arsenal of phage resistance mechanisms that are, in different combinations, widespread in bacteria and archaea. Actually, this evolutionary dialogue has shaped the genomic trajectory of microbial chromosomes, and previous studies have shown that phage resistance mechanisms often cluster within genomic defense islands (Makarova *et al*, 2011). One such defense island includes *pglZ*, which has been implicated in phage resistance as defined by phage growth limitation (Pgl) in *Streptomyces coelicolor* (Chinenova *et al*, 1982). The

Pgl genotype consists of *pglWXYZ*, an operon encoding a serine/threonine kinase (PglW), an adenine-specific methyltransferase (PglX), an ATP-binding P-loop protein (PglY) and an alkaline phosphatase (PglZ).

Investigating the occurrence of PglZ-containing cassettes in the genomes of ~1,500 bacteria and archaea, Goldfarb and collaborators establish that this phage resistance system is phylogenetically widespread, subject to horizontal gene transfer, and occurs in approximately 10% of microbial genomes. In the majority of cases, *pglZ* appears in a six-gene cassette (which they call BREX), which includes *pglZ* itself (phosphatase), *pglX* (methyltransferase) as well as *brxABCL*, that encodes an RNA-binding anti-termination protein (BrxA), an unknown protein (BrxB), an ATP-binding protein (BrxC) and a protease (BrxL). Further comparative analyses established six major BREX types based on gene sequence, content and number, all containing *pglZ*.

In order to establish a direct correlation between the BREX genotype and a phage resistance phenotype, the authors insert the *Bacillus cereus* BREX system (Fig 1) into the *Bacillus subtilis* chromosome and confirm functional transcription in two operons (*brxABC-pglX* and *pglZ-brxL*). Next, they use a range of lytic and lysogenic phages for viral infection. Results show up to 10⁻⁵ reduction in efficiency of plaquing, illustrating a relatively high level of BREX-dependent phage resistance against both virulent and temperate phages.

Mechanistically, the authors reveal that BREX is distinct from the canonical Pgl system, as it provides phage resistance prior to the first round of infection. Because neither the release of newly generated phages, nor the integration of phage into the

host chromosome was detected, they conclude that the BREX system provides resistance against both lytic and lysogenic phages. The authors rule out abortive infection and also show that BREX does not prevent phage adsorption, but rather blocks phage DNA replication.

Remarkably, using Pac-Bio sequencing, the authors demonstrate that the host DNA is widely m6A methylated at the fifth position of a non-palindromic 5'-TAGGAG-3' hexamer motif, whereas the phage DNA is not, perhaps allowing the host to distinguish viral from chromosomal DNA and presumably prevent self targeting. Indeed, deletion of the *pglX* gene that encodes a DNA methylase results in abrogated resistance, strongly suggesting that methylation of the host chromosome is required for BREX-mediated viral defense.

Southern blot analyses of total cellular DNA reveal replication of phage DNA in BREX-lacking cells, but not in BREX-encoding cells. The authors exclude a mechanism of action akin to R-M, as there was no sign of phage DNA cleavage or degradation, suggesting that BREX is involved in inhibiting phage replication and propagation rather than degradation of viral DNA. The authors also test whether BREX can confer resistance against plasmid DNA uptake; results indicate that there is a mild effect on episomal plasmid uptake, and no impact on integrative plasmid transformation. Other experiments reveal the ability of some phages to evade or circumvent BREX-encoded resistance, in a manner reminiscent of what was recently established for bacteriophage escape of CRISPR-Cas adaptive immune systems (Bondy-Denomy *et al*, 2013), further illustrating the arms race nature of host-phage dynamics.

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Figure 1. Bacteriophage exclusion.

(A) Invasion of a bacterial host by a temperate phage (left, red) and a lytic phage (purple, right). In the absence of an effective defense response, invasion will result in integration of the viral DNA in the host chromosome, whereas the DNA of the lytic viruses will be transcribed, translated and replicated, eventually leading to a new generation of viruses and lysis of the host cell. (B) Bacterial hosts that possess the bacteriophage exclusion (BREX) system are resistant to many (but not all) temperate and lytic phages. Different types of BREX systems are encoded by conserved gene clusters (in case of type-1 BREX from *Bacillus cereus*, two operons: *brxABC-pglX* and then *pglZ-brxL*). The well-conserved core of the BREX system includes three enzymes with (predicted) functionality: an ATP-binding P-loop protein (PglY/BrxC, C), an alkaline phosphatase (PglZ, Z) and a methyltransferase (PglX, X) that specifically methylates TAGGAG. The *B. cereus* BREX system has been demonstrated to inhibit the integration of temperate phages, as well as the replication (and proliferation) of lytic phages (Goldfarb *et al*, 2015).

Overall, this study provides the crucial proof of concept that BREX constitutes a novel phage resistance system, which is distinct from previously characterized viral defense mechanisms, both genetically and phenotypically. This defense system allows phage absorption and DNA integration into

the host cell, but precludes viral replication in a methylation-dependent manner, unravelling a role for epigenetics in bacterial virus resistance. Though present in only 10% of microbial genomes, this innate immune system is phylogenetically widespread. Future studies should determine the molecular

underpinning of BREX-encoded immunity in bacteria and shed light on the biochemical processes that drive host chromosome methylation-dependent phage resistance. Additional studies should assess whether this system has a fitness cost and whether it coexists with other defense systems, notably R-M, Abi and CRISPR. The findings presented in Goldfarb *et al* advance our understanding of the role of phage resistance systems in the arms race between bacterial communities and their viral predators and potentially open new avenues for engineered phage resistance in bacteria.

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