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BACTERIAL PHYSIOLOGY

Phage injection establishes CRISPR immunity

> CRISPR machinery obtains most spacers from the first region of the phage genome that is injected

The CRISPR–Cas system protects bacteria from invaders by acquiring short DNA sequences from infecting viruses. These sequences (spacers) protect against subsequent infection by mediating the cleavage of invading DNA by Cas nuclease. The details of spacer acquisition are currently unclear. Modell *et al.* now show that CRISPR–Cas systems capture spacers from the free ends of injected viral DNA and that these spacers protect against subsequent infection with the same phage.

Captured spacer sequences are integrated into the CRISPR locus and transcribed into CRISPR RNA guides. During subsequent phage infection, the RNA guide binds to the corresponding site on the invading DNA and directs cleavage. To study spacer acquisition, the authors supplied *Staphylococcus aureus*, which lacks a CRISPR system, with a plasmid that expresses the *Streptococcus pyogenes* type II-A CRISPR–Cas locus, in which all spacer sequences were removed. Newly incorporated spacers were detected and quantified by PCR amplification and sequencing. When this system was used to examine autoimmune spacer acquisition from the bacterial chromosome there was an adaptation hotspot seen around the chromosomal terminus, which is the site of free DNA ends during replication. This suggests that there is a preference for spacer acquisition from free DNA ends.

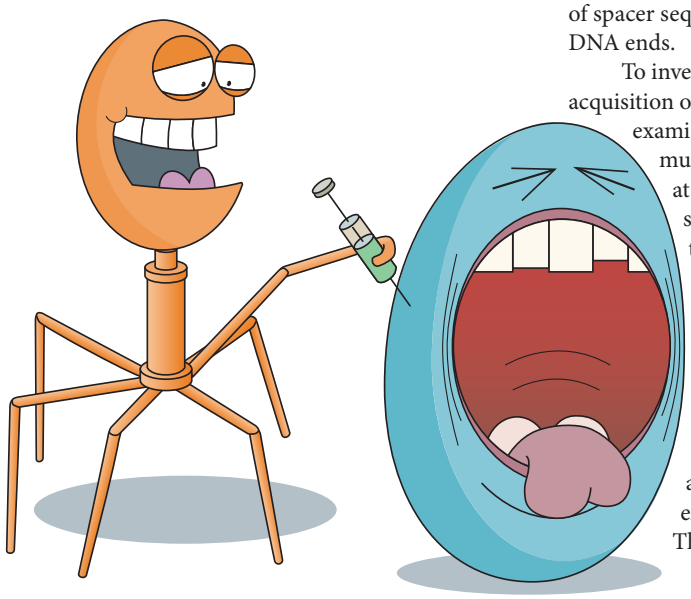
Next, the authors investigated spacer acquisition after infection with the λ-like lytic Φ12γ3 bacteriophage. During the first infection cycle, most spacers were acquired from a 13 kb region that is flanked by a *cos* site (where free DNA ends are generated during genome packaging) and the first upstream *chi* site (a recombination hotspot). Thus, CRISPR immunity to Φ12γ3 is also generated through the acquisition of spacer sequences from free viral DNA ends.

To investigate when spacer acquisition occurs, the authors examined a panel of Φ12γ3 mutants that are impaired at different life cycle stages following injection: *polA* mutants cannot continue the lytic cycle following DNA injection, *terS* mutants cannot package DNA and mutants with a deletion in the genes that encode holin and lysin fail to produce extracellular virions. These mutants induced similar patterns of spacer acquisition, which suggests that capture occurs during or just after genome injection. Next, the authors infected bacteria with a mutant that has an inverted *cos* site (with an inverted pattern of injection). In contrast to wild-type phages, most spacers were acquired from the region downstream of the *cos* site. These data suggest that the CRISPR machinery obtains most spacers from the first region of the phage genome that is injected.

To investigate the effect on subsequent immunity, bacteria were generated that contained spacers from different locations in the Φ12γ3 genome. Cells that survived infection were found to be enriched in spacers that targeted the genomic region that was first injected into the host. The opposite pattern was observed when cells were infected with the mutant that had an inverted *cos* site. Finally, bacteria that lack a CRISPR–Cas system were mixed with bacteria that contained spacers derived from the region upstream of *cos*; these mixed cultures were able to recover and survive after phage challenge. These results suggest that CRISPR–Cas systems that are adapted to target early injected phage sequences provide better immunity to viruses.

In summary, type II CRISPR–Cas systems capture free DNA ends during phage injection, halting subsequent infection before the lytic cycle begins.

Shimona Starling



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