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BACTERIAL GENETICS

A CRISPR sense of self

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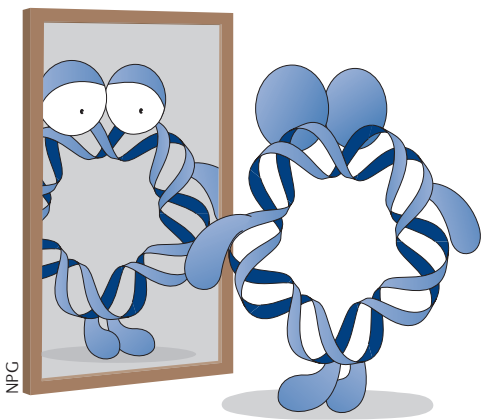
CRISPR–Cas is a prokaryotic adaptive immune system that provides sequence-specific protection against invading plasmids and phages. Adaptive immunity is conferred by the integration of DNA sequences (called spacers) from an invading element into the CRISPR array, which is transcribed into short CRISPR RNAs that guide Cas proteins to degrade complementary foreign DNA on subsequent exposures. The molecular details of the CRISPR–Cas pathway have largely been elucidated, but how Cas proteins distinguish 'self' DNA from 'non-self' DNA during spacer acquisition has been unclear. Sorek, Qimron and colleagues now show that spacer acquisition occurs predominantly at replication termination sites, which are more abundant in highly proliferative foreign DNA.

The authors sequenced the CRISPR array of the type I-E system in *Escherichia coli* to monitor spacer acquisition *in vivo*. The particular strain that was used lacks all of the *cas* genes on its chromosome, but those encoding Cas1 and Cas2 (which mediate spacer acquisition) are plasmid borne, which enables spacer acquisition while downstream interference is blocked. Using this system, they identified events in which the system failed to discriminate between self and non-self DNA by detecting spacers that were derived from the bacterial chromosome.



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A strong bias in spacer acquisition was revealed, in which sequences known to be prone to replication fork stalling were hot-spots for integration into the CRISPR locus. This finding was confirmed by experiments that showed increased spacer acquisition from a synthetic chromosomal locus that is prone to stalling. Importantly, the concentration of replication forks (and hence the susceptibility to form stalled forks) in plasmids is known to be greater than that in chromosomes, suggesting that the CRISPR system inherently favours plasmid DNA.

As stalled replication forks are major sources of DNA double-strand breaks (DSBs), the authors reasoned that spacers, whether acquired from the bacterial chromosome or from plasmid DNA, might be derived from degradation intermediates that are generated by the RecBCD exonuclease during DSB repair. Indeed, in strains lacking *recB* or *recC*, a substantial reduction in spacer acquisition was observed. As RecBCD also recognizes phage genomes, RecBCD degradation intermediates from phages could also explain the bias of the CRISPR system for acquiring phage DNA.

RecBCD terminates its activity when it reaches an octameric sequence called Chi, which suggests that these sites limit spacer acquisition. Analysis of the CRISPR array sequencing data confirmed that this was consistently the case. Notably, Chi sites are strongly enriched in chromosomal DNA compared with plasmid or phage DNA, which suggests that RecBCD degradation intermediates are derived more frequently from foreign DNA than from self DNA, which would bias the CRISPR system towards the selection of foreign DNA.

Overall, these data support a model in which spacers are acquired when Cas1 (which has been shown to interact with RecB and RecC) and Cas2 bind to DNA fragments that are readily generated from plasmid and phage DNA. This bias for non-self DNA seems to be mediated by the higher number of replication forks in plasmid DNA, the susceptibility of phage DNA to RecBCD and the low number of Chi sites in both plasmid and phage DNA.

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ORIGINAL RESEARCH PAPER Levy, A. *et al.* CRISPR adaptation biases explain preference for acquisition of foreign DNA. *Nature* **520**, 505–510 (2015)