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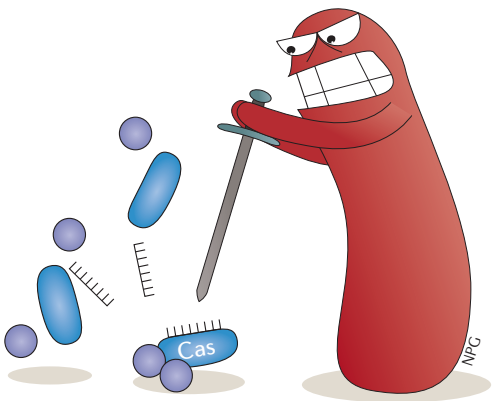
BACTERIAL GENETICS

Defeating the drawbacks of CRISPR

> Plasmid evasion of CRISPR immunity is driven by host mutations

In addition to providing protection against lytic phages, the CRISPR–Cas (clustered, regularly interspaced short palindromic repeats–CRISPR-associated proteins) systems of bacteria and archaea can prevent the acquisition of beneficial, horizontally transferred DNA. Here, Marraffini and colleagues investigate this drawback of CRISPR–Cas immunity and find that the most common strategy used to overcome immunity against beneficial plasmids involves either the inactivation or the loss of CRISPR–Cas loci.

CRISPR loci contain repetitive DNA motifs separated by spacer sequences that are complementary to target sequences found in invading phages or plasmids. CRISPR transcripts are processed into small CRISPR RNAs, which — together with the Cas proteins — target and cleave subsequent invaders. Although the presence of anti-phage spacers has clear advantages, the widespread occurrence of anti-plasmid spacers in bacteria is difficult to reconcile with the fact that the uptake of exogenous genetic elements is often crucial for prokaryotic adaptation and survival.



To investigate how bacteria cope with this drawback of CRISPR immunity, the authors carried out conjugation experiments, in which the staphylococcal pG0400 plasmid (which encodes mupirocin resistance) was transferred to mupirocin-sensitive *Staphylococcus epidermidis* RP62A. The CRISPR–Cas system of this neomycin-resistant strain contains a spacer that is specific for pG0400, so by plating the bacteria on agar containing both mupirocin (to select for the plasmid) and neomycin (to select against plasmid donors), only those transconjugants that evade CRISPR immunity and acquire the plasmid are selected.

The authors found that approximately half of the 111 transconjugants tested had either an inactivating mutation (such as a spacer deletion, a transposon insertion or a single nucleotide deletion or substitution) in the CRISPR array, and the remaining transconjugants had a deletion of the entire CRISPR–Cas locus. Furthermore, fluctuation experiments showed that CRISPR mutants emerged spontaneously and at a high rate (between 10^{-4} and 10^{-3} per cell per generation).

If the inactivation or loss of CRISPR–Cas loci constitutes a real mechanism that is used by natural bacterial populations to acquire beneficial plasmids, then the observed mutations and deletions are expected to confer little or no fitness cost. To test this hypothesis, pairwise growth competition experiments were performed between wild-type *S. epidermidis* RP62A and each of six transconjugants, which represented the full spectrum of the isolated CRISPR escape mutants. Although some of the mutants were less fit than the wild type, several were either as fit or even more fit, which suggests that CRISPR-deficient mutants are likely to emerge in the natural environment.

Finally, considering that target mutation is the most common mechanism for phage evasion of CRISPR immunity, it was surprising that none of the transconjugants contained mutations in the pG0400 target. Computer simulations based on experimental parameters indicated that plasmid mutations occur at much lower rates than host mutations that deactivate or delete CRISPR, thus explaining the absence of target mutations. If this is a general feature of CRISPR loci, then it suggests that the 'arms race' between CRISPR loci and their targets is driven by two distinct processes: plasmid evasion of CRISPR immunity is driven by host mutations, whereas phage evasion is driven by target mutations.

Collectively, these data suggest that CRISPR–Cas systems are in a constant state of flux owing to the conflicting positive and negative selective pressures for immunity against lytic phages and beneficial plasmids, respectively. The authors propose that this could explain the large variation in the presence, the number and the functions of CRISPR–Cas loci within and between bacterial species.

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ORIGINAL RESEARCH PAPER Jiang, W. *et al.* Dealing with the evolutionary downside of CRISPR immunity: bacteria and beneficial plasmids. *PLoS Genet.* **9**, e1003844 (2013)