Trends in Microbiology

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Spotlight

CRISPR-Cas and restriction–modification team up to achieve long-term immunity

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Bacteria have been shown to harbor a growing arsenal of various defense systems against phages. Maguin *et al*. have uncovered how two of the most frequent defense systems interact: the clustered regularly interspaced short palindromic repeats-Cas (CRISPR-Cas) system recycles by-products of the restriction–modification (RM) system to increase bacterial defense in the long run.

To accommodate the ever-evolving diversity of phages, bacteria have developed multiple antiviral strategies. In recent years, many novel antiphage mechanisms have been described. However, their ecological importance is only starting to be understood. For example, a bacterium encodes, on average, five antiphage systems [1], raising the possibility of interactions between defense mechanisms upon phage infection. Recent works started to explore these potential interactions, unveiling the existence of complex immune strategies in bacteria. For example, an abortive infection phenotype can be triggered either following a failed CRISPR-Cas immune response in type III CRISPR-Cas systems [2] or retron-mediated in case of impaired RecBCD nuclease activity [3].

RM and CRISPR-Cas are the most common antiphage systems, present in respectively 83% and 39% of prokaryotic genomes [1]. Consequently, many genomes encode both systems, raising the question of their interactions. It was previously observed that heterologous expression of type II RM in *Streptococcus thermophilus* was compatible with the presence of endogenous type II CRISPR-Cas and provides additive protection against phage infection [4]. The study demonstrated that the two lines of defense are important to prevent phage-escape mechanisms. Following these observations, a second study with the same experimental set-up showed that the systems are not only compatible but are also synergistic [5]. More precisely, phage inactivation by RM systems facilitates the acquisition of new spacers from the inactive phage by the CRISPR-Cas system [5]. However, the molecular mechanism of how the synergy is achieved remained unexplored.

In a recent study [6], Maguin and colleagues determined the molecular mechanism of the cooperation between RM and CRISPR-Cas: cleavage of viral DNA by the RM generates double-strand breaks (DSBs), producing substrates for the acquisition of spacers in CRISPR-Cas systems. This adaptation allows a long-term CRISPR-based immunity required to bypass RM-resistant mutants (Figure 1).

A first step in the study was to establish a novel experimental set-up in *Staphylococcus aureus* to evaluate the different antiviral responses: RM only, CRISPR-Cas only, RM and CRISPR-Cas together. The RM-based antiviral response, while efficient, is quickly overcome by phages that escape the antiviral system through methylation of their genome. In the experimental set-up, it takes about 4 h for the phage to escape RM. Strains with CRISPR-Cas systems – which, originally, did not include a spacer targeting the infecting phage – present no resistance phenotype compared to the negative control without systems. However, when both CRISPR-Cas and RM systems are present in the strain, a long-term antiviral response (growth comparable to control after 10 h) is observed.

The authors then tested the hypothesis proposed by Hynes and colleagues [5] which suggests that phage inactivation would drive acquisition of spacers by a type II CRISPR-Cas. Using defective phage mutants that are unable to replicate, they demonstrate that spacer acquisition does not occur in the absence of RM under these conditions. Indeed, the presence of a type I RM system, irrespective of whether the phage can replicate or not, is a determinant in the acquisition of a new spacer. The cleavage of the phage by the RM system is mechanistically essential for spacer acquisition.

To better understand this phenomenon, the authors followed up on the discovery that DSBs and free DNA ends are key factors in the acquisition of new spacers [7–9]. First demonstrated through the role of the DNA repair complex RecBCD in adaptation [9], DSBs generated by the AddAB repair machinery [8] or Cas9 were also shown to promote adaptation [7]. As RM systems create DSBs when cleaving DNA, the authors hypothesized that newly acquired spacers come from regions of the phage DNA that are cleaved by RM. The initial type I RM tested cleaves many targets (>25) at random distance from the recognition site, making the signal too difficult to interpret. To overcome this, the authors introduced a type II RM that cuts at a precise location and in one position. Using this new CRISPR–RM combination, they first reproduced the previous results that both antiphage systems are required for long-term survival. They showed that the newly acquired spacers originate from the neighborhood of the RM restriction site. To further validate this observation, the authors engineered the phage to modify the sequence of the restriction site and showed that the sequences of the acquired spacer changed accordingly. Finally, they demonstrated that AddAB contributes to the adaptation process notably by restricting further the viral DNA once cleaved by the RM, providing additional DSBs for spacer

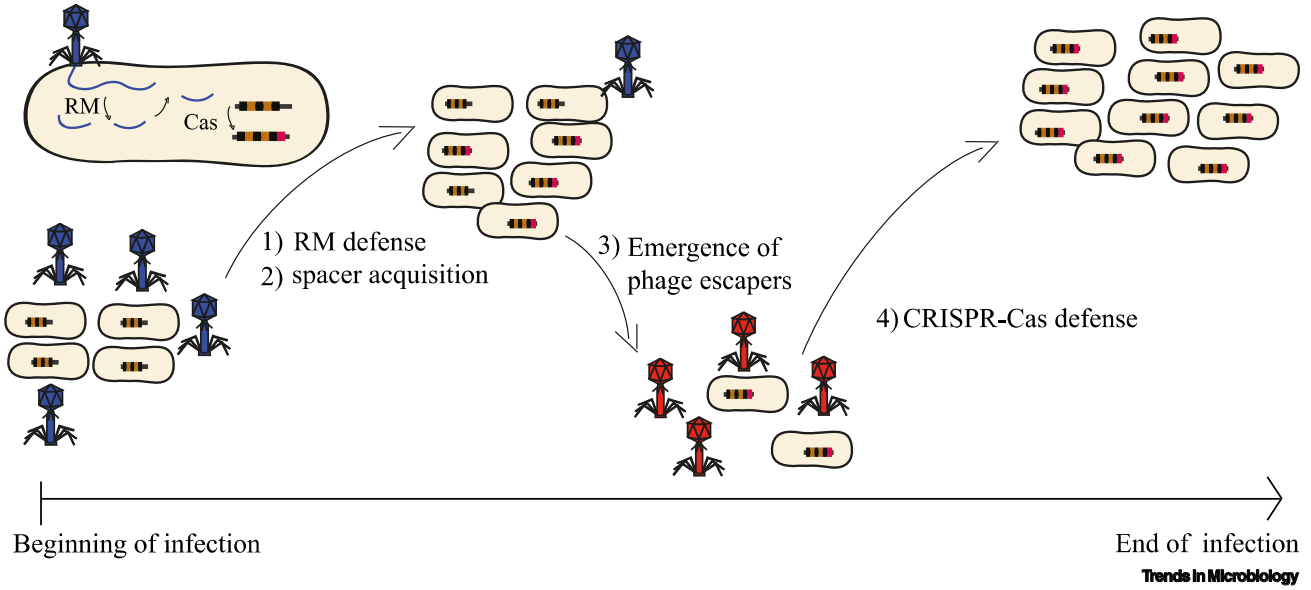

Figure 1. Clustered regularly interspaced short palindromic repeats-Cas (CRISPR-Cas) recycles restriction–modification (RM) by-products to achieve long-term immunity. A bacterial population with an RM and a CRISPR is infected by a phage susceptible to the RM (blue). RM cleaves incoming phage DNA, providing short-term immunity (1). Double-strand breaks (DSBs) generated by RM cleavage are used by the Cas machinery to incorporate a novel spacer specific to the blue phage (red spacer) (2). Shortly, phage mutants escaping RM arise (red) (3); only cells that acquired a CRISPR spacer specific to the phage survive (CRISPR-Cas still targets RM-escapees). This novel spacer is the base for long-term CRISPR-based immunity (4).

acquisition further off the restriction site. Overall, these results led the authors to conclude that the DSBs generated by the RM are indeed used for spacer acquisition.

The work of Maguin *et al*. uncovers a new mechanism of acquisition of long-term immunity against phages. While demonstrated on type II CRISPR-Cas and type II RM, the molecular details of the mechanism (DSBs generated by RM) suggest that RM-derived spacer acquisition could be widespread across other RM/CRISPR combination types. Given that 92% of CRISPR-Cas systems are found in genomes where an RM is present [1], this could represent a major mode of acquisition of CRISPR spacers in the wild. Furthermore, this paper demonstrates how synergy between two different defense systems is achieved. Interestingly, the by-products of the most common of the two antiphage systems (RM) are harnessed by the less frequent one (CRISPR-Cas). The relative frequency of systems across prokaryotes could serve as an indicator of the direction of interactions between systems. Thus, it is possible that the molecular mechanisms of RM and CRISPR-Cas, the most common antiphage systems, are harnessed by less frequent antiphage systems to achieve antiviral immunity. Finally, it was recently shown that antiphage system interactions are not limited to cooperation. Indeed, an example of competition between two types of RM has been reported [10], demonstrating that the complexity of interactions between antiphage systems is only starting to be uncovered.

Declaration of interests

No interests are declared.

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References

1. Tesson, F. *et al.* (2021) Systematic and quantitative view of the antiviral arsenal of prokaryotes. *bioRxiv* Published online September 3, 2021. https://doi.org/10.1101/2021.09.02.458658v2
2. Niewoehner, O. *et al.* (2017) Type III CRISPR-Cas systems generate cyclic oligoadenylate second messengers to activate Csm6 RNases. *Nature* 548, 543–548
3. Millman, A. *et al.* (2020) Bacterial retrons function in anti-phage defense. *Cell* 183, 1–11
4. Dupuis, M.-È. *et al.* (2013) CRISPR-Cas and restriction–modification systems are compatible and increase phage resistance. *Nat. Commun.* 4, 2087
5. Hynes, A.P. *et al.* (2014) Adaptation in bacterial CRISPR-Cas immunity can be driven by defective phages. *Nat. Commun.* 5, 4399
6. Maguin, P. *et al.* (2022) Cleavage of viral DNA by restriction endonucleases stimulates the type II CRISPR-Cas immune response. *Mol. Cell* 82, 907–919.e7
7. Nussenzweig, P.M. *et al.* (2019) Cas9 cleavage of viral genomes primes the acquisition of new immunological memories. *Cell Host Microbe* 26, 515–526.e6
8. Modell, J.W. *et al.* (2017) CRISPR–Cas systems exploit viral DNA injection to establish and maintain adaptive immunity. *Nature* 544, 101–104
9. Levy, A. *et al.* (2015) CRISPR adaptation biases explain preference for acquisition of foreign DNA. *Nature* 520, 505–510
10. Birkholz, N. *et al.* (2022) A mobile restriction–modification system provides phage defence and resolves an epigenetic conflict with an antagonistic endonuclease. *Nucleic Acids Res.* 50, 3348–3361