IMMUNE EVASION

First evidence of prokaryotic RNAi?

DOI:

10.1038/nrmicro1671

URLs

Streptococcus thermophilus
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genome&retmode=Retrieve&dopt=Overview&list_uids=13177

Results reported in a recent *Science* paper provide the first experimental evidence to support the hypothesis that prokaryotes possess a defence system that might be analogous to eukaryotic RNA interference (RNAi).

Clustered regularly interspaced short palindromic repeats, or CRISPRs, were first identified in prokaryotic genomes almost 10 years ago. Each element comprises a direct repeat of 28–40 bp separated by a unique 25–40-bp spacer, and they are typically arranged in arrays that can contain up to 250 elements. It had previously been noted that there was a correlation between the number of spacers in CRISPR loci and resistance to phage infection, and this led to the suggestion that CRISPR loci might be involved in defence against phage attack. This hypothesis was further supported by *in silico* analyses showing that some CRISPR spacer sequences are homologous to phage or plasmid genes, and in a large-scale bioinformatics study of CRISPR loci and CRISPR-associated (*cas*) genes published last year, a possible molecular mechanism for this prokaryotic RNAi system was proposed by comparing the predicted functions of the Cas proteins to the eukaryotic RNAi machinery.

Until now, however, experimental evidence to support this hypothesis was lacking. Barrangou and colleagues began by analysing what happened to the CRISPR loci in a phage-sensitive *Streptococcus thermophilus* strain during challenge with two different phage. Nine of the phage-resistant mutants generated had 1–4 new spacers, and sequence

analysis revealed that these spacer sequences were similar to the challenge phage. These results indicate not only that resistance to phage challenge is mediated by the integration of phage DNA, but also that this is a rapid and dynamic process. This process was further probed by experimentally adding or deleting spacers and assessing the corresponding resistance, and the results confirmed the relationship between spacer content and phage resistance, although it was found that the spacers might require a specific genetic context to confer resistance. Finally, two *cas* genes, *cas5* and *cas7*, were inactivated in an *S. thermophilus* phage-resistant mutant. The *cas5*-deletion mutants lost their resistance and the authors speculate, based on its amino-acid sequence, that the Cas5 protein might be a nuclease.

The *cas7*-deletion mutants retained their resistance, and the authors speculate that the Cas7 protein might be involved in the synthesis and/or insertion of new spacers.

Proving that prokaryotes possess an adaptive 'immune' response that is analogous to eukaryotic RNAi will be a long process. This work gives us a tantalizing glimpse of what lies ahead.

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ORIGINAL RESEARCH PAPER Barrangou, R. et al. CRISPR provides acquired resistance against viruses in prokaryotes. *Science* **315**, 1709–1712 (2007)

FURTHER READING Makarova, K. S. et al. A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. *Biol. Direct* **1**, 7 (2006) | Bolotin, A. et al. Clustered regularly interspaced short palindromic repeats (CRISPRs) have spacers of extrachromosomal origin. *Microbiology* **151**, 2551–2561 (2005)