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MICROBIAL GENETICS

CRISPR memories of RNA

> "the acquisition of RNA spacers by type III CRISPR systems may enable the targeting of parasitic RNA species"

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CRISPR–Cas systems are widespread among prokaryotes and provide a form of adaptive immunity. Short segments of invading DNA are captured and integrated as interspersed spacers within CRISPR arrays, providing a heritable memory that directs the targeting of matching nucleic acid sequences for digestion. In a paper published in *Science*, Silas *et al.* now report that a subset of CRISPR systems has the ability to acquire spacers not only from DNA but from RNA as well.

Previous genomic analyses had shown that, in some CRISPR systems, the CRISPR-associated protein Cas1, which catalyses spacer integration (along with Cas2), is fused to a putative reverse transcriptase (RT). Silas *et al.* carried out phylogenetic analyses of the genes encoding RT–Cas1 fusions, and found that all classifiable fusions belonged exclusively to the type III class of CRISPR systems.

To explore the ability of RT–Cas1 to facilitate spacer acquisition, the authors focused on the type III-B system from the melanogenic marine bacterium *Marinomonas mediterranea* strain MMB-1. Overexpression of RT–Cas1 in MMB-1 resulted in the new acquisition of spacers within the native CRISPR array. Interestingly, these spacers were enriched for sequences from highly transcribed genes, and this enrichment was abolished by deletion of the RT domain. Such an RT-dependent bias is consistent with spacer acquisition involving the reverse transcription of RNA. The authors assessed this possibility in MMB-1 through the introduction of a plasmid encoding genes that contained self-splicing introns. The subsequent detection of newly integrated spacers corresponding to spliced transcripts confirmed the ability of RT–Cas1 to facilitate the acquisition of spacers from RNA.

To investigate the mechanism of spacer acquisition, the authors incubated purified RT–Cas1 and Cas2 with DNA or RNA oligonucleotides, a CRISPR DNA substrate, and deoxynucleoside 5′-triphosphates (dNTPs) to enable reverse transcription. These assays revealed that either DNA or RNA oligonucleotides could be ligated directly to the CRISPR substrate. Moreover, the sensitivity of a ligated RNA oligonucleotide to RNase H digestion demonstrated its existence as part of an RNA–DNA hybrid, consistent with reverse transcription of the ligated RNA to generate cDNA.

The ligation of both RNA and DNA oligonucleotides to the CRISPR substrate was abolished by a mutation in the Cas1 active site, but only the ligation of RNA oligonucleotides was abolished by deletion of the RT domain, consistent with the RT-dependent bias towards highly transcribed regions that was observed *in vivo*.

Finally, using radiolabelled dNTPs to facilitate the detection of cDNA, the authors tested whether an RNA oligonucleotide integrated into a CRISPR array could be reverse transcribed by the RT–Cas1–Cas2 complex *in vitro*. The cDNA products detected were consistent with the reverse transcription of integrated RNA primed by the 3′ end of the CRISPR DNA of the opposite strand. The authors suggest that these cDNAs may represent an intermediate in the acquisition of a fully integrated double-stranded DNA spacer.

The team concludes that the acquisition of RNA spacers by type III CRISPR systems may enable the targeting of parasitic RNA species, such as RNA phages, and may contribute to immune responses towards highly transcribed regions of DNA phages and plasmids via an interference system targeting DNA, RNA or both. In addition, the authors highlight the possibility that RNA spacer acquisition could also occur in species that have seperately encoded RT and Cas1 proteins.

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ORIGINAL ARTICLE Silas, S. *et al.* Direct CRISPR spacer acquisition from RNA by a natural reverse transcriptase-Cas1 fusion protein. *Science* http://dx.doi.org/10.1126/science.aad4234 (2016)
FURTHER READING Sontheimer, E. J. & Marraffini, L.A. CRISPR goes retro. *Science* **351**, 920–921 (2016)

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