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Poster #30: Phage-encoded single-guide RNAs subvert CRISPR-Cas9 immunity

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Bacteriophages are relentless innovators in the molecular arms race with bacteria. To evade adaptive immune systems like CRISPR-Cas—which utilize RNA-guided nucleases to cleave viral genomes—phages have evolved a vast array of counter-defences. However, despite this evolutionary creativity, the known anti-CRISPR (acr) repertoire remains almost exclusively composed of proteins. Here, we uncover a fundamentally distinct counter-defence strategy: phage-encoded viral single-guide RNAs (vsgRNAs) that disable type II CRISPR-Cas immunity through RNA-guided rewiring of Cas9 function. We show that vsgRNAs, encoded in *acr*-associated genomic contexts and predicted to inhibit diverse type II-A and II-C systems, act through a two-tier inhibitory mechanism. First, they capture the Cas9 apoenzyme and divert it from endogenous phage-targeting crRNAs. Second, by means of a truncated guide region, they redirect Cas9 to bind but not cleave its own promoter, thereby silencing cas9 transcription. Strikingly, phages generated vsgRNAs by co-opting the same guide and promoter target site used by extended tracrRNAs that autoregulate cas9 expression. In this way, vsgRNAs enable phages to simultaneously disable ongoing interference and restrict renewed production of the immune effector. We further show that phages similarly co-opted small CRISPR-associated RNAs (scaRNAs) targeting the cas9 promoter. These findings reveal that phages hijack intrinsic CRISPR–Cas autoregulatory logic to repurpose a bacterial immune nuclease for RNA-mediated immune silencing, uncovering a previously unrecognized class of anti-CRISPR effectors and a new principle of prokaryotic virus–host conflict.

Reference #1

Reference #2

Reference #3

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