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Cas13 Helps Bacteria Play Dead when the Enemy Strikes

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How RNA-targeting CRISPR-Cas13 functions as a phage defense system has been mysterious. Recently in *Nature*, Meeske et al. (2019) demonstrate that Cas13 provides potent immunity to dsDNA phages without cutting their genome. By sensing phage transcripts and destroying RNA nonspecifically to arrest the cell into dormancy, Cas13 provides herd immunity.

Clustered regularly interspaced short palindromic repeats and their associated proteins (CRISPR-Cas) represent a large and diverse family of adaptive bacterial immune systems. CRISPR-Cas systems protect against mobile genetic elements such as plasmids and bacteriophages (phages). CRISPR-Cas systems contain a collection of spacer sequences, which are DNA elements derived from previously encountered foreign DNA. CRISPR RNAs (crRNAs) are generated from these spacer elements that guide an encoded nuclease to cleave complementary phage nucleic acids. To capture their broad evolutionary origins and mechanisms of action, six distinct types of CRISPR-Cas systems have been described in the literature to date and the most recently discovered family, Type VI, is the only one that exclusively targets RNA (Abudayyeh et al., 2016). Upon sequence-specific cleavage of target transcripts, the type VI nuclease Cas13 is activated to cleave other RNA species *in trans* without sequence specificity, so-called "collateral damage." This non-specific activity has been previously observed *in vitro* and in a heterologous expression system in *E. coli* (Abudayyeh et al., 2016).

Despite the development of many Cas13-based technologies for mammalian application (Cox et al., 2017), limited attention has been given to Cas13's native biology. Writing in *Nature*, Meeske et al. (2019) address many fundamental questions about Cas13 function (Meeske et al., 2019). Given that spacers encoded by type VI CRISPR-Cas systems appear to be derived from DNA phages, the authors reasoned that these are a target in nature. To determine whether Cas13 can provide immunity against a double-stranded DNA (dsDNA) phage and which genes make the most effective targets, the authors designed a library of 41,276 spacers tiled every 2 nucleotides across each strand of a DNA *Listeria* phage genome. *Listeria* is a natural host of CRISPR-Cas13 and was transformed with this pooled library and infected with the target phage. The authors observed that Cas13 protected against the DNA phage with a strand bias for complementarity between the crRNA and the target messenger RNA (mRNA). Interestingly, targeting of either essential or non-essential genes, as well as early or late genes, led to comparable levels of crRNA enrichment, demonstrating that immunity does not depend on the target gene's function. Using RNA-seq, the authors found that immune activity is proportional to the transcription levels of the target RNA and identified Cas13 non-specific RNA cleavage of host and phage mRNAs, by mapping 5′ RNA ends with sequencing. Interestingly, DNA replication and late transcripts were not detected when a phage early gene was targeted, suggesting that important early gene products and/or host factors were depleted. Targeting of late transcripts allowed phage DNA levels to accumulate to similar levels as an untargeted phage, but productive lysis was still prevented by Cas13. This demonstrates that detection of the target transcript arrests cellular function, though immunity is independent of phage DNA replication.

The authors then hypothesized that immunity is mediated via cellular dormancy due to widespread RNA cleavage. They found a significant growth defect during immunity and also utilized a controlled non-phage system where target transcription could be induced. When the target was transcribed, cells entered growth arrest but were still viable and could recover when target transcription was halted, even after 9 hours. In phage experiments, this immune activity led to strong reductions in phage titer and enabled "herd immunity," preventing an engineered escaper phage or an untargeted phage from replicating (see Figure 1).

In this paper, the authors successfully addressed many of the open questions surrounding CRISPR-Cas13 as an immune system. Importantly, this work was done in a system that naturally encodes CRISPR-Cas13, a critical strength, as much CRISPR-Cas biology is done in non-native model organisms. With this publication, the authors convincingly demonstrate that Cas13-based immunity protects from dsDNA phages, remarkably protecting bacterial populations from the emergence of CRISPR-resistant escaper phages. It is curious to consider the parallels between type VI and type III CRISPR-Cas, another system that the Marraffini group has shown prevents the replication of escaper mutant phages (Pyenson et al., 2017). Typically, Cas proteins use a short protospacer adjacent motif (PAM) directly adjacent to their target to differentiate between the spacer found in the host CRISPR array, which lacks the PAM, and a viral protospacer target. By targeting RNA, which is transcribed in only one orientation, rather than DNA, neither the type III or type VI systems need a PAM to discriminate self from foreign spacer matches. The lack of a PAM denies phages an effective site of escape mutation and greatly diminishes the production of escaper phages.

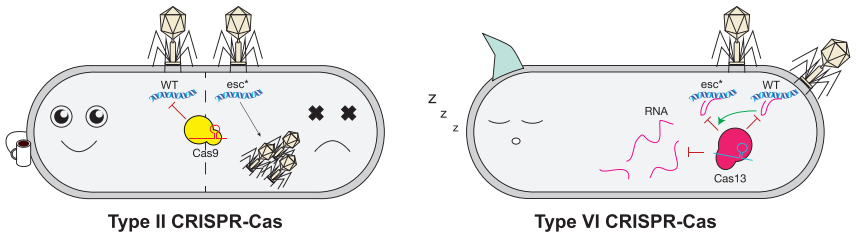

Figure 1. Type VI CRISPR-Cas Systems Induce Dormancy to Block Phage Replication
CRISPR-Cas9 provides potent immunity against targeted phages (left), but escaper mutations can allow phages to evade immune activity and replicate successfully. CRISPR-Cas13 destroys RNA non-specifically after detection of its target RNA, preventing the replication of wild-type and escaper phages.

This work answers essential questions of type VI CRISPR-Cas biology while opening several avenues for future investigation. The authors show that removal of the target transcript can alleviate the dormancy; however, it is unknown whether higher Cas13 and/or target expression make dormancy irreversible. It is also unclear whether dormancy reversal occurs during infection, should a phage manage to lysogenize and silence the target gene. Additionally, it is interesting to consider what the host response might be to this degradation. Are some transcripts, perhaps structured ones, protected from degradation or is there selective translation under this stress? An analogous dormant state is the cold shock response in *E. coli*, in which most translation is completely inhibited, but certain gene products continue to be produced to facilitate exit from cold shock (Zhang et al., 2018). It is unknown whether any similar programs exist during Cas13-mediated dormancy. This enigma regarding exit from dormancy complicates the acquisition of spacers. The authors hypothesize that spacers can be acquired from defective phages that inject their genome into cells but are unable to carry out infection. Alternatively, they propose that there might indeed be a mechanism to exit dormancy and that spacers represent memories of previous infections that were survived. Lastly, we consider how phage diversity might affect Cas13 efficacy. Do phages have mechanisms to protect or modify their RNA to prevent detection? And what effect, if any, does mRNA structure have on initial *cis* Cas13 recognition and cleavage?

With this foundational inroad into type VI CRISPR-Cas biology, Meeske et al. (2019) contribute a clarified understanding of type VI CRISPR-Cas in nature, distinguishing it from all other characterized CRISPR-Cas systems that target DNA. These data put Type VI CRISPR-Cas into a functional category with previously described abortive infection mechanisms where bacteria commit suicide or cleave RNA during infection (Parreira et al., 1996; Fineran et al., 2009). Each system has its own mechanism to specifically detect phage infection, and here Type VI CRISPR-Cas uses base pairing. Future work focused on the broad diversity of Cas13 orthologs and assessing their function in native systems, where possible, will undoubtedly reveal interesting new biology.

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