

## **CRISPR-Cas10 immunity in staphylococci: Mechanisms and applications**

Staphylococci are prevalent skin-dwelling bacteria that are also leading causes of antibiotic-resistant infections. The viruses that infect these organisms (phages) are potent antimicrobials that are being explored as alternatives to conventional antibiotics. However, staphylococci possess multiple layers of anti-phage defense systems that threaten to undermine the effectiveness of therapeutic phages. In addition, phages are replete with genes of unknown functions, which could cause unknown downstream side-effects. Therefore, gaining a detailed understanding of therapeutic phages as well as the systems bacteria use to defend against them are both critical to the success of whole-phage antimicrobial applications. We are currently investigating mechanisms and applications of the anti-phage defense system known as CRISPR-Cas (Clustered regularly interspaced short palindromic repeats-CRISPR-associated). These systems use small CRISPR RNAs (crRNAs) and Cas nucleases to detect and destroy phages and other nucleic acid invaders. Many staphylococci possess Type III-A CRISPR-Cas systems, also known as CRISPR-Cas10. Although recent work has defined the roles of many of its Cas proteins, nothing is known about how this system integrates with other cellular pathways to mount an effective defense. Our recent work demonstrates that CRISPR-Cas10 employs nucleases from the RNA degradosome to ensure robust immunity. Based on this observation, we have developed a general two-step approach to edit genomes of virulent staphylococcal phages using CRISPR-Cas10 to counter-select for desired phage mutants. Altogether, the mechanistic insights into CRISPR-Cas10 immunity and its phage-editing applications are expected to enable the rational design of phage-based antimicrobials that effectively combat drug-resistant staphylococci.