Loss-of-Function Mutations in Csx27 Reveal Pore Activity Is Required for CRISPR–Cas13b Antiviral Defense

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CRISPR–Cas systems enable bacteria to defend against viral infection through RNA-guided nucleases and accessory proteins, though the functions of many accessory components remain unclear. This project examines the role of the membrane protein Csx27 in CRISPR–Cas13b antiviral defense using structure-guided point mutations designed to disrupt pore activity without altering operon structure or Cas13b expression. Wild-type and mutant constructs were tested in E. coli using plaque assays and infection-dependent growth curves. While wild-type Csx27 strongly suppressed phage replication and induced growth arrest, pore-disrupting mutants failed to provide robust defense and closely resembled empty vector controls, with one mutant exhibiting partial activity. These findings demonstrate that Cas13b RNA cleavage alone is insufficient for full immunity and that Csx27 pore activity is required to link viral recognition to effective cellular defense. Because mutations at both pore-facing and interface residues impaired function, the results further support a model in which Csx27 acts through an oligomeric membrane pore whose assembly or stability is important for antiviral defense.

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