Exploring the Mechanism of Translation-Dependent Downregulation of Cas12a mRNA

Jorge L. Rodriguez[1], Alexander Talaie[2], Theresa Astmann[1], Nicole D. Marino[1,2]

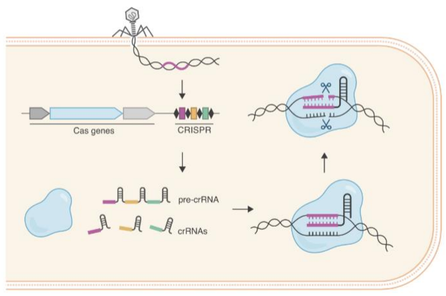
1 Department of Pathobiology, University of Pennsylvania, Philadelphia, PA; 2 Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA

Abstract

Bacteria have evolved multiple defense systems, including CRISPR-Cas, to cleave the DNA of phage and mobile genetic elements (MGE). In turn, phages have evolved anti-CRISPR (Acr) proteins that use novel and co-opted mechanisms to block DNA binding or cleavage. We previously found that an anti-CRISPR protein (AcrVA2) unexpectedly inhibits Cas12a biogenesis by binding conserved and functionally important amino acids in the Cas12a N-terminal polypeptide and degrading it. However, the mechanism of underlying Cas12a mRNA destruction remains mysterious. Here, we show that co-expressing AcrVA2 and Cas12a in a simplified *invitro* transcription-translation assay does not trigger Cas12a mRNA degradation, indicating that other bacterial factors are required. Inhibition of Rho terminase, which mediates premature transcriptional termination, does not affect Cas12a mRNA downregulation, suggesting that Rho is not involved. We then tested the role of transfer-messenger RNA (tmRNA), which rescues ribosomes stalled during translation and triggers degradation of the truncated mRNA and polypeptide via trans-translation. Deletion of tmRNA restores fragments of Cas12a mRNA, indicating a role in Cas12a downregulation. Continuous co-expression of AcrVA2 recruits mysterious host factors to truncate Cas12a mRNA during translation and then targets the truncated Cas12a mRNA and protein for destruction via trans-translation.

Background

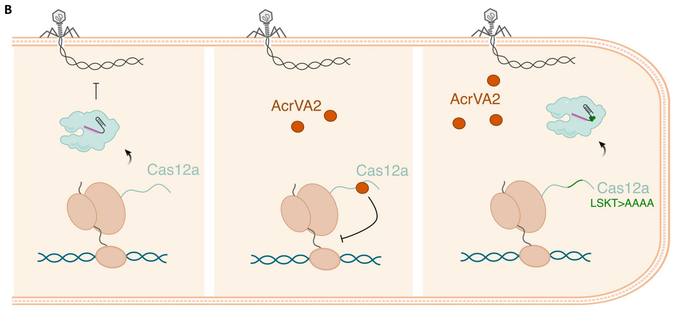
A



CRISPR-Cas systems protect bacteria from viral (phage) infections.

Marino, Nature Methods, 2020

B

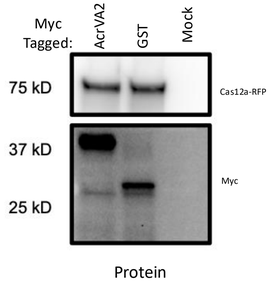


AcrVA2 interferes with Cas12a biogenesis by binding to the N-terminal polypeptide chain and downregulating its mRNA.

Marino, bioRxiv, 2023

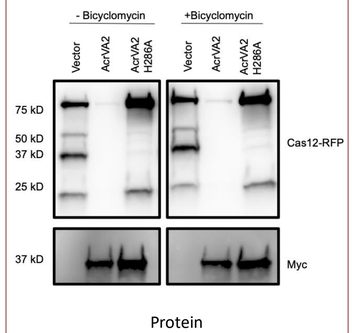
Results

AcrVA2 does not downregulate Cas12a *in vitro*



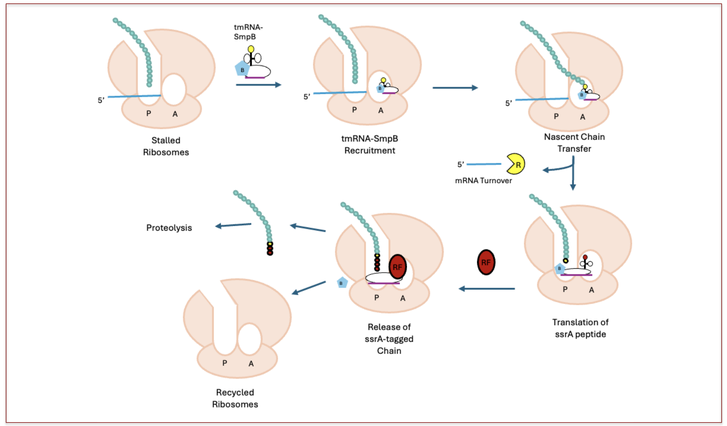
Mysterious bacterial factors are required for Cas12a downregulation

Rho Terminase does not appear to be required for Cas12a downregulation



Bicyclomycin inhibits Rho terminase from causing premature transcriptional termination

tmRNA rescues stalled ribosomes through trans-translation



Acknowledgments

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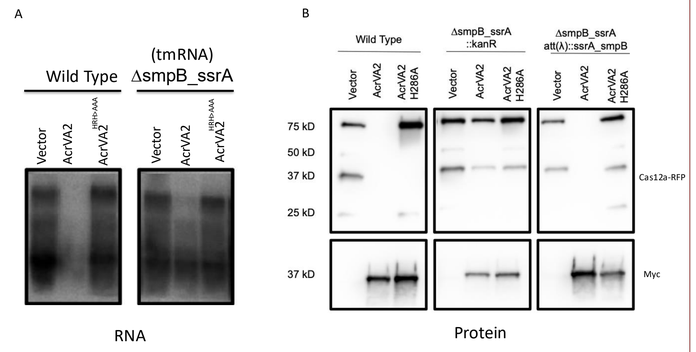
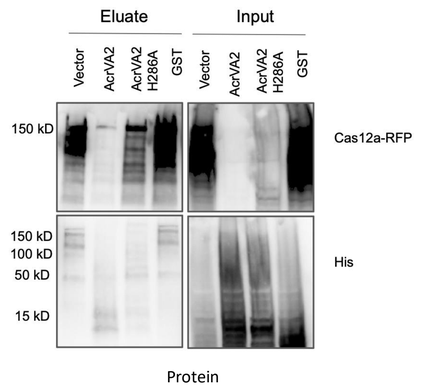
Results

tmRNA is required for full downregulation of Cas12a mRNA by AcrVA2



Cas12a mRNA fragments are restored when tmRNA is absent

AcrVA2 co-immunoprecipitates with His-tagged fragments



Future Steps

- Confirm with complementation that tmRNA is required for Cas12a downregulation
  - Determine if Cas12a is degraded through trans-translation by AcrVA2
  - Use other approaches to find other bacterial factors required for Cas12a downregulation