Investigating the Mechanism of Novel Anti-CRISPR in Type I-E CRISPR System

Kaiya Vierra[1], Nathaniel Burman[2], Royce Wilkinson[2], Blake Wiedenheft[2]

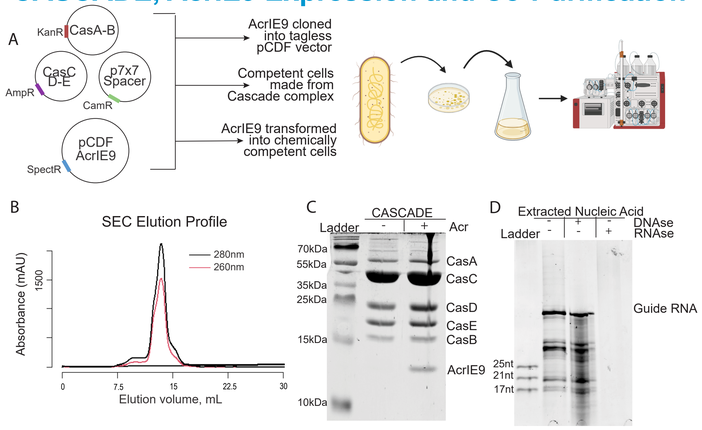
[1]*Department of Chemistry and Cell Biochemistry, Montana State University, Bozeman, MT 59717, USA*
[2]*Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT 59717, USA*

ABSTRACT

Viruses that infect bacteria (bacteriophages) are the most abundant biological entity on earth, causing more than10^{23} infections every second[1,2]. As a result of this predation, prokaryotes have evolved diverse defense systems, including CRISPRs (Clustered Regularly Interspersed Short Palindromic Repeat), which use RNA-guided protein complexes to seek and destroy viral nucleic acids, blocking infection[3]. In response, bacteriophages have evolved countermeasures called Anti-CRISPR (Acr) proteins that block host immunity and rescue infection. Acrs are diverse and studies suggest that there is a unique Acr adapted to block most, if not all subclasses of CRISPR systems[4]. Here we present our investigation of a novel Acr that inhibits a Type I-E CRISPR complex termed CASCADE. To provide a molecular understanding of how AcrIE9 blocks CASCADE-mediated defense, we have employed Cryo-Electron Microscopy (Cryo-EM), a cutting-edge structural biology technique.

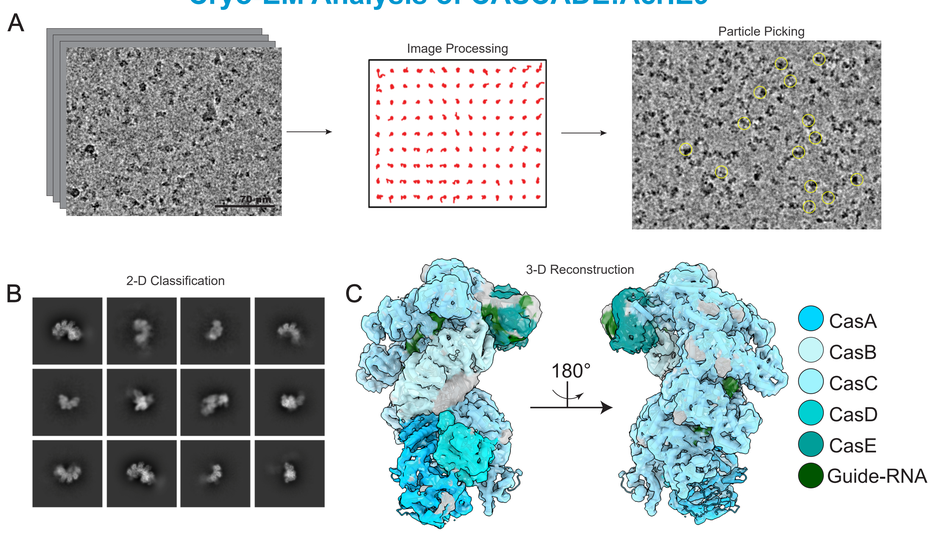
RESULTS

CASCADE, AcrIE9 Expression and Co-Purification



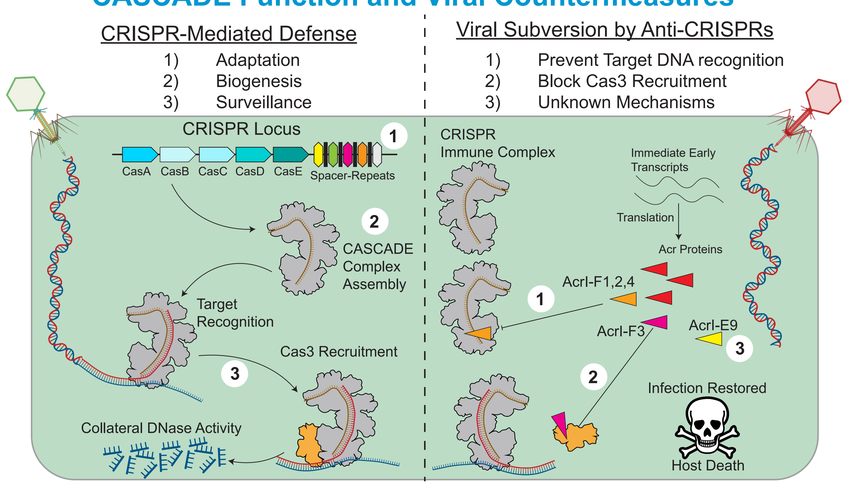
A) Plasmids used for expression of CASCADE and AcrIE9 with a schematic of the purification workflow. Affinity-tagged CasC was used as bait to pull down the assembled CASCADE complex and bound AcrIE9 B)SEC Elution profile of pulldowns reveals a monodisperse peak with a calculated molecular weight of 420kDa C)15% SDS PAGE of the main peak in B) reveals all subunits of the CASCADE complex are present and that AcrIE9 co-purifies with the complex. D) 12% Urea PAGE of extracted RNA from purifiedCASCADE:AcrIE9 reveals mature guideRNA remains bound to the complex.

Cryo-EM Analysis of CASCADE:AcrIE9



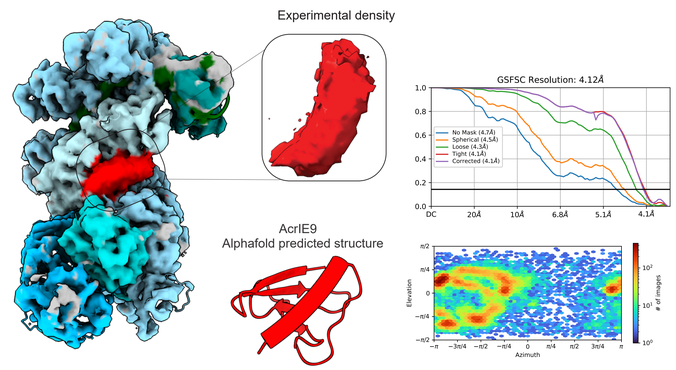
A) Selected micrograph showing a field of Cascade:AcrIE9 particles. After motion correction and CTF-estimation, particles were picked and extracted for analysis. Picked particles are highlighted with yellow circles.
B) Representative 2D classes of particles selected for 3-D reconstruction
C) Density map of the Cascade:AcrIE9 complex colored according to subunit with previously determined structures docked into the map (PDB: 5CD4).

CASCADE Function and Viral Countermeasures



CRISPR-Mediated Defense can be broken down into three universally conserved steps: 1) Adaptation, 2) Biogenesis, and 3) Surveillance. Once assembled, the RNA-guided CRISPR complex searches for viral nucleic acids complimentary to the RNA-guide. Virally encoded Acr proteins have evolved to block CRISPR systems through a variety of different mechanisms so infection can be restored in the host.

Resolving Density For AcrIE9



≈4.5Å resolution reconstruction of the CASCADE:AcrIE9 complex. Putative density for AcrIE9 is weak, possibley due to orientation bias or sampleheterogeneity. FSC curve and viewing angle distribution plots shown.

DISCUSSION

This work represents significant progress towards determining the mechanism by which AcrIE9 blocks CASCADE-mediated immunity using a structural approach. Although we have not yet determined a high-resolution structure of CASCADE bound to AcrIE9, the expression and purification of the CASCADE:AcrIE9 complex have now been optimized. Current Cryo-EM analysis suggests that AcrIE9 may function through interactions with CasB that prevent conformational changes in CasA necessary for target DNA recognition[5]. To achieve the resolution necessary to model AcrIE9 we are currently testing additional image analysis strategies and plan on collecting an additional Cryo-EM dataset in the future.

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