Determining the Mechanism of Detecting and Destroying Invading DNA

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Hypothesis

We hypothesize that select areas of the Cse1 subunit of Cascade are critical in the recruitment of Cas3 to the Cascade complex. To test this hypothesis, we have generated structure guided mutations and tested the impact of these mutations on immune function. Some of the mutations completely abrogate immune function while others have no measurable impact.

Methods

Hydrogen deuterium exchange (HDX)
•We use hydrogen deuterium exchange to investigate areas of Cascade that become more accessible for HDX upon binding with dsDNA. These areas suggest that those areas are interaction sites for Cas3.

Site directed Mutagenesis
•We create mutants of Cascade based on HDX data that allow us to investigate specific areas of Cascade and their role in Cas3 recruitment.

Plasmid Curing Assays
•We use plasmid curing assays to determine how specific Cascade mutations impact the function of the immune system.

Cascade Structure

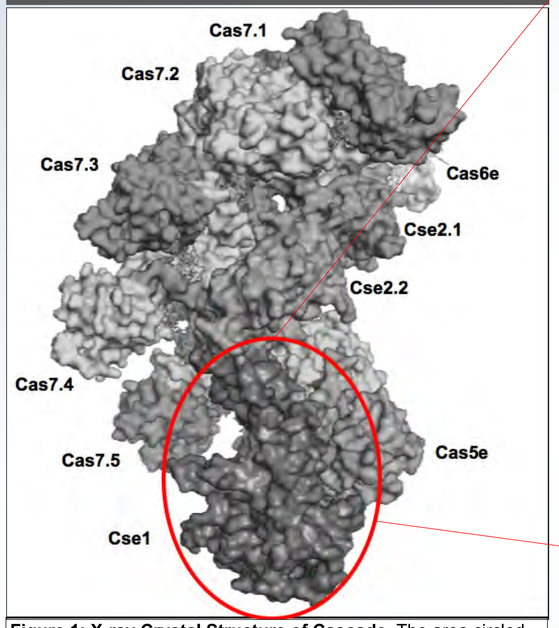

Figure 1: X-ray Crystal Structure of Cascade. The area circled in red shows the Cse1 subunit of Cascade. This area has been found to undergo a conformational change around the time of Cas3 recruitment, therefore making it an area of interest.

Abstract

Bacteria and Archaea have evolved RNA-guided adaptive immune systems called CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas (CRISPR associated) that provide protection against invading genetic elements. The Type 1-E CRISPR-Cas system of *Escherichia coli* utilizes Cascade (CRISPR associated complex for antiviral defense), a surveillance complex that is composed of eleven protein subunits and a CRISPR-derived RNA (crRNA) guide. Cascade searches the intracellular environment for complementary nucleic acid targets and binds to those that match the crRNA guide. Once bound, Cascade undergoes conformational changes, signaling for the recruitment of a trans-acting Cas3 nuclease/helicase. Cas3 can then bind to the Cascade complex and degrade the invader DNA. It is known that Cas3 interacts with the Cse1 subunit of the Cascade complex, however the mechanism of Cas3 recruitment remains unknown. Our aim is to determine the mechanism of Cas3 recruitment to better understand how foreign DNA is degraded.

Cse1 Areas of Interest

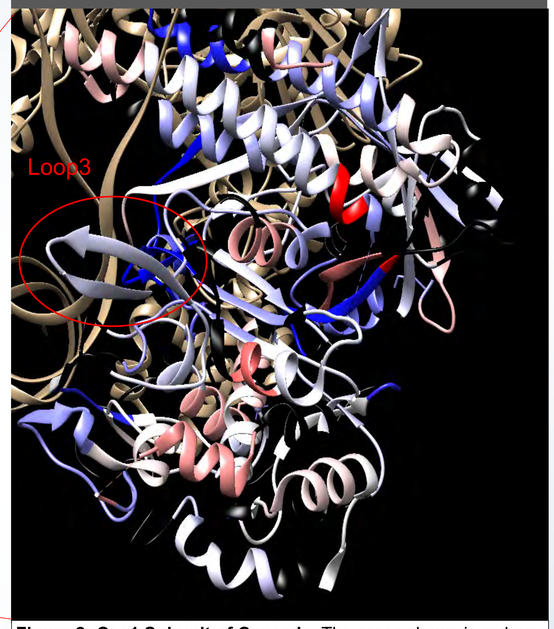

Figure 2: Cse1 Subunit of Cascade. The areas shown in red are areas more available for HDX when Cascade is bound to dsDNA which indicates that they might be interacting with Cas3. The areas in blue display low amounts of HDX in unbound Cascade. The loop circled in red is Loop3. Loop3 is one area of the Cse1 complex that we are interested in exploring. Plasmid curing assay results of Loop3 are shown in Figures 4 and 5.

Results of Plasmid Curing Assay

Active Immune System

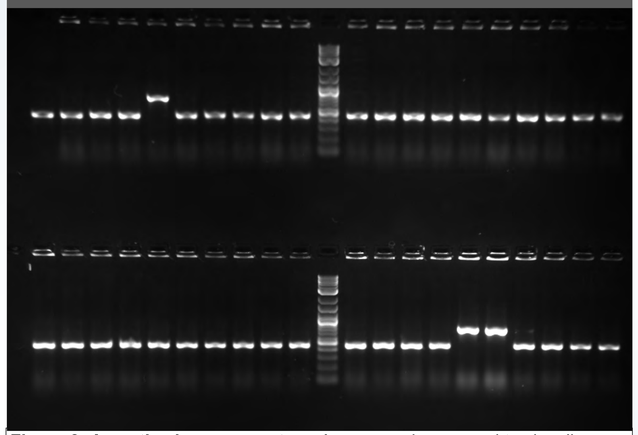

Figure 3: An active immune system. Agarose gels are used to visualize plasmid curing results. The larger bands are the target plasmids and the smaller bands are the non-target plasmids. The target bands in this experiment contain a 350bp sequence insert that matches the sequence of the crRNA. The target plasmid should be destroyed in an active immune system, but the non-target should remain un-recognized. This image shows forty experiments in which only three of the forty target plasmids were not destroyed.

Broken Immune System (Loop3)

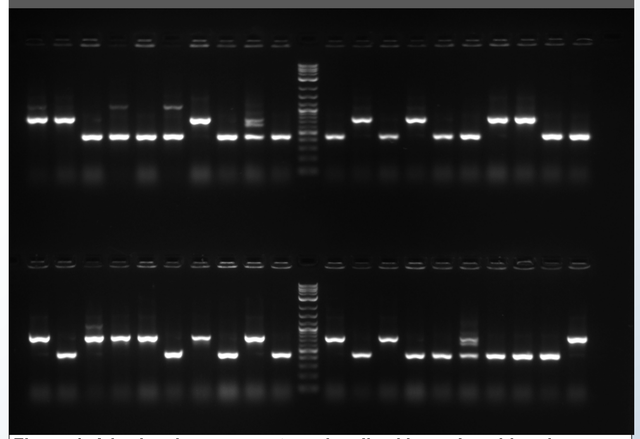

Figure 4: A broken immune system visualized by a plasmid curing assay. Agarose gels are used to visualize plasmid curing results. The smaller bands are the non-target bands and the larger bands are the target bands. The target bands in this experiment contain a 350bp sequence insert that matches the sequence of the crRNA. In a working immune system, the target plasmid is destroyed, leaving only non-target bands. In this Loop3 deletion result, we see that out of forty experiments, eighteen of the target plasmids were not destroyed.

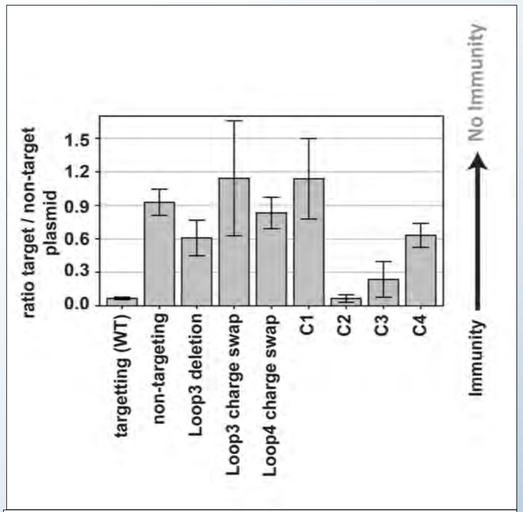

Figure 5: Impact of Cascade Mutants on Immune Function. Each gray bar represents the average Target/Non-Target plasmid ratio from three separate experiments for each mutant. The black error bars show the standard error of the mean for each mutant. Mutants that have gray bars closer to zero indicate a more active immune system while mutants with gray bars closer to one represent more defects in immune function.

Conclusions

We used Hydrogen Deuterium Exchange (HDX) to locate areas on the Cse1 subunit of the Cascade complex that become more flexible after dsDNA binding. We hypothesized that areas that become more accessible after binding dsDNA were involved with the recruitment of Cas3. We designed mutants at these locations to determine their role in adaptive immunity. Plasmid curing experiments reveal specific regions of Cascade that are critical for immune system function.

Future Directions

After we have completed all of the plasmid curing assays, we will purify successful mutant proteins and perform binding and cleavage assays to test if the mutant Cascades can still bind to dsDNA and, if Cas3 can still be recruited to the Cascade/DNA complex to degrade the invader DNA.

Acknowledgements

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