Determined the requirements for acquisition of new CRISPR immunity elements in *Escherichia coli*

**What is CRISPR?**

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is an adaptive immune system found in roughly 50% of bacteria and 90% of archaea. This system works through three stages: adaptation, biogenesis, and interference (Figure 1). Adaptation, the focus of this project, is when bacteria incorporate new immunity elements ("spacers") from invading DNA molecules. Biongenesis allows spacers to be transcribed into CRISPR RNAs (crRNAs). Interference occurs when a crRNA is bound by Cascade, a complex of CRISPR-associated (Cas) proteins. Complex searches for DNA that has sequence complementary to the crRNA. If an invading DNA molecule, such as a plasmid or bacteriophage genome, is targeted by Cascade, the foreign genetic material will be destroyed, rendering the invader incapable of causing harm.

**Adaptation**

Adaptation, sometimes called "spacer acquisition", occurs when the cell identifes foreign genetic material and obtains a new segment of DNA ("spacer") from the invader, to incorporate into its genome. There are two types of adaptation: naive and primed. Naive adaptation incorporates a spacer from a newly encountered invader whereas priming, the focus of this project, acquires another unique spacer from a previously encountered invader. Each type of adaptation requires different proteins to occur. Primed acquisition additionally requires an existing spacer against an already encountered invader. Adaptation ultimately results in the addition of one new spacer and one repeat to the CRISPR array. Many cis- and trans-acting factors likely affect spacer acquisition, but not all have been identified.

**The Leader Sequence**

The leader sequence is a region of DNA directly upstream from the CRISPR array and often separates the array and the CRISPR genes. The leader sequence has been shown to be lost by recombination and several other species, which implies some importance to the leader sequence. Many of these positions are conserved between related species. It is likely that the conserved base pairs are the most important elements. The conserved positions could act as binding sites for one or more DNA binding proteins. However, these results only showed what elements were necessary for priming spacer acquisition, not why. These results should be further explored to try and determine the actual purpose of each element and why it is important to acquisition of new CRISPR immunity elements.

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**Results**

All three CNC CRISPR I leader sequence mutants were unable to acquire new spacers via primed adaptation after 5 hours of growth. However, all mutants were able to acquire new spacers in the CRISPR II array, which had a wild-type leader sequence. Although this study does not identify the precise base pairs in the leader sequence necessary for adaptation, base pairs in both the 40-50 bp and 50-60 bp regions are important. Many of these positions are conserved between related species. It is likely that the conserved base pairs are the most important elements. The conserved positions could act as binding sites for one or more DNA binding proteins. However, these results only showed what elements were necessary for priming spacer acquisition, not why. These results should be further explored to try and determine the actual purpose of each element and why it is important to acquisition of new CRISPR immunity elements.

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