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Molecular Relatedness of Two Distinct Type IV CRISPR-Associated (Cas) Proteins

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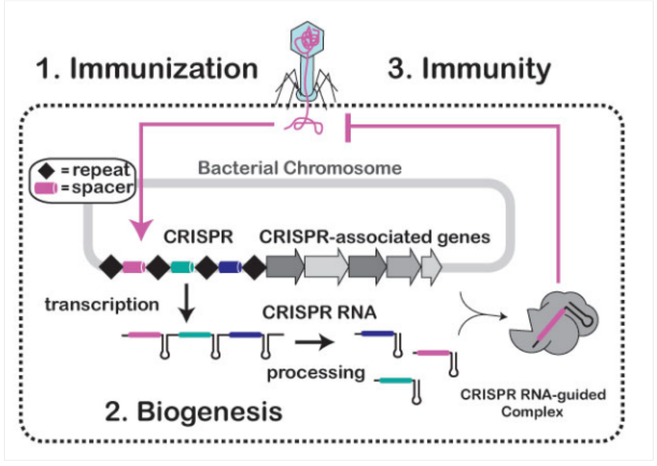
Introduction

CRISPR-Cas systems are prokaryotic adaptive immune systems. Bacteria use CRISPR systems as a defense against foreign nucleic acid invasion such as phage infection.

- CRISPR systems are **diverse** (Type I-VI) and include many different subtypes
- **Type IV** CRISPR system biology is poorly understood–our lab's research is dedicated to studying this system type which could lead to breakthroughs in genomic editing tools
- **Cas6-IV** is an **endoribonuclease** (protein capable of cleaving/cutting up DNA and RNA)
- **DinG** is a putative **helicase** (protein that unwinds duplexed/double-stranded DNA)

By studying Cas protein function in Type IV CRISPR systems, we gain a better understanding of the system mechanisms and biological significance. Here, molecular and structural phylogenetic studies were conducted to investigate two Cas proteins.



Methods

The amino acid sequences of various Cas6 and DinG proteins were aligned by:

1. A multiple sequence alignment of DinG proteins via Clustal Omega and creation of an alignment-based tree via FigTree.
2. Cas6 sequences were aligned according to known (PDB) and predicted (PSI-PRED) secondary structure.

Figure 1. Three-step mechanism of adaptive immunity of a typical CRISPR system (left). A cartoon depiction of a Type IV CRISPR system locus, including all associated proteins, and spacers stored within a CRISPR cassette to facilitate target sequence recognition (above).

Results

DinG sequences belonging to Type IV CRISPR systems are phylogenetically dissimilar to DinG sequences not involved in adaptive bacterial immunity (Figure 2). Cas6 proteins are diverse in sequence but have conserved secondary structure motifs to enable binding and cleavage of crRNA substrates (Figure 3).

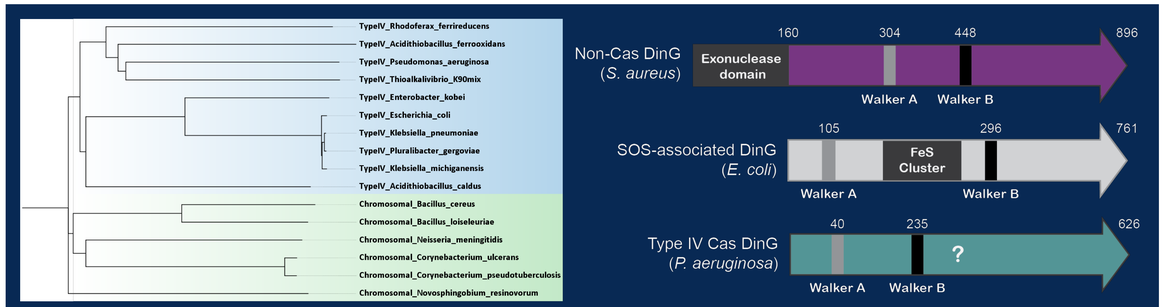


Figure 2. Phylogenetic tree detailing the relatedness between Cas DinG and non-Cas DinG. A clear evolutionary divergence is observed between the two types (left). Domain organization of three DinG homologues. *S. aureus* DinG is known to function as an endonuclease, SOS-DinG in *E. coli* is a helicase that is upregulated under genomic stress, and the characterization of Type IV Cas DinG is unknown (right).

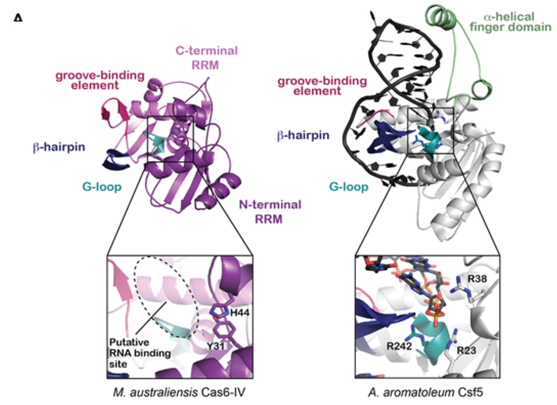


Figure 3. *Ma*Cas6-IV and *Aa*Csf5 structurally aligned.

Conclusions

The data collected from this study allow us to continue our investigation into Type IV CRISPR systems, specifically:

1. Expression, purification, and characterization of a Type IV CRISPR-associated DinG protein
2. Comparison of the crystal structure of Type IV Cas DinG to non-Cas DinG

These aims, in addition to other projects being pursued by our lab, will give us a deeper understanding of bacteria biology and provide the basic research required for breakthroughs in genomic editing tools.

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