12-5-2022

Expressing and Purifying Type IV CRISPR Accessory Proteins

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Introduction

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas (CRISPR-associated) systems are adaptive immune systems that defend bacteria and archaea against phages, plasmids, and other mobile genetic elements.

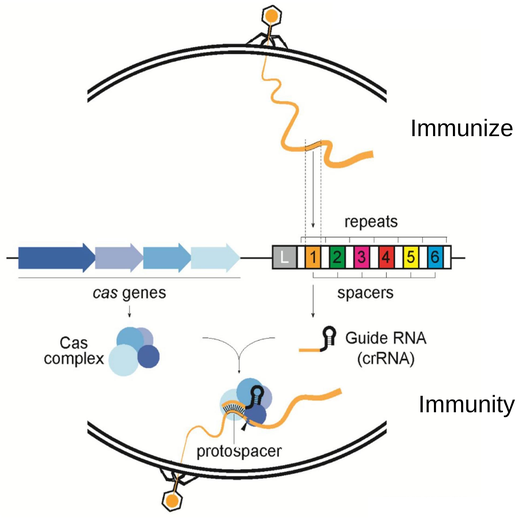


Figure 1: CRISPR systems immunize cells by integrating short pieces of viral DNA into one side of the CRISPR region. The CRISPR is transcribed into small RNA molecules that are transcribed into Cas-proteins. Form complexes that use rnRNA to recognize nucleic acid to protect from mobile genetic elements[2].

Diversity CRISPR Systems

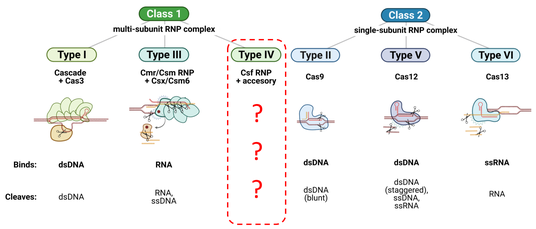
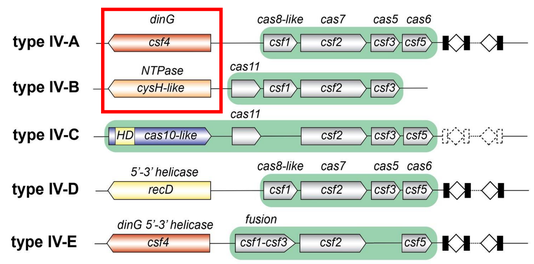


Figure 2: CRISPR systems are extremely diverse and are grouped into two classes. Within the two classes they are further separated into 6 types, and over 33 subtypes.

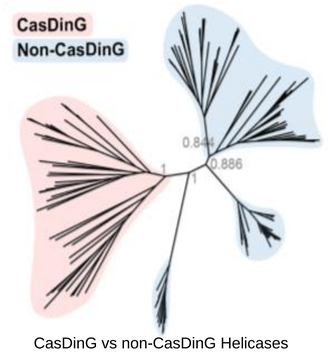
Comparison of Type IV CRISPR Systems



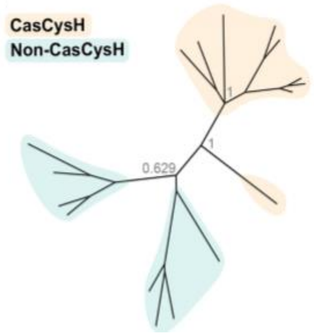
Each Type IV CRISPR system contains a subtype-specific gene and is hypothesized to be essential for function. The Type IV-A system encodes ATP-dependent 5'-3' DNA helicase called CasDinG, while the Type IV-B systems encode a putative pyrophosphatase named CasCysH. The molecular mechanisms of many CRISPR systems, including Type IV-V, have not been determined. Type IV-B CRISPR systems are unique in that they lack a CRISPR array and do not have a Cas protein with an obvious nuclease, suggesting Type IV-B systems function differently than other CRISPR-Cas systems. The focus of my work has been to determine the function of these two proteins.

Results

DinG and CysH Proteins associated with type IV CRISPR systems are distinct



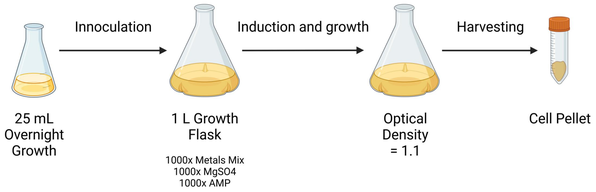
CasDinG vs non-CasDinG Helicases



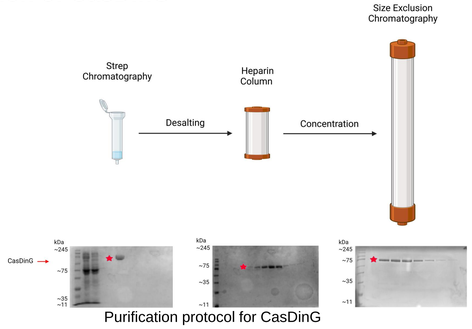
CasCysH vs non-CasCysH

Phylogenetic analysis of CasDinG and CasCysH proteins showed that they were distinct form the other DinG and CysH proteins.

Expression and Purification of CasDinG



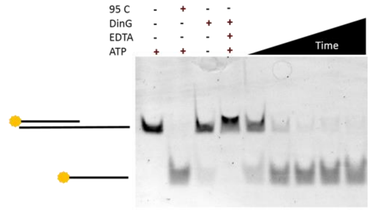
Expression protocol for CasDinG



Purification protocol for CasDinG

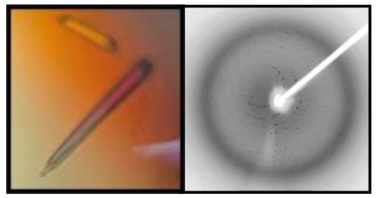
CasDinG was expressed in HMS174 *E. coli* cells. The growth, expression, and purification of the protein were optimized. CasDinG is purified through strep chromatography, ion exchange, and size exclusion chromatography.

CasDinG is a Helicase



Helicase assay in the presence of a DNA 5' overhang[1].

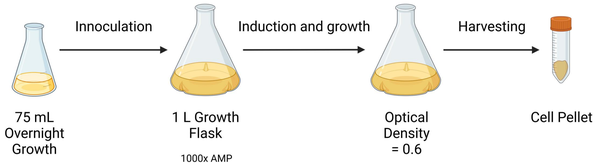
X-Ray Crystallography of CasDinG



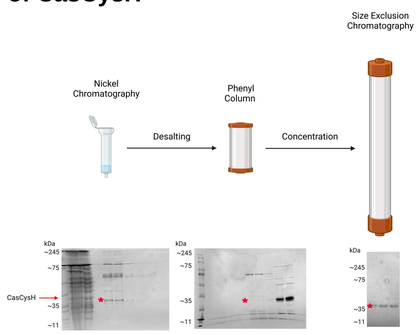
Crystals (left) and diffraction pattern (right) obtained from the X-ray crystallography of CasDinG[1].

A helicase assay was used to help confirm the helicase activity of CasDinG. Additionally, X-ray crystallography was used to help determine the structure of CasDinG.

Expression and Purification of CasCysH



Expression protocol for CasCysH



Purification protocol for CasCysH

CasCysH was expressed in Nico21 *E. coli* cells. The growth, expression, and purification were optimized. CasCysH is then purified in three steps that involve nickel chromatography, a phenyl column, and size exclusion chromatography .

Future Directions

Future directions of this work with CasCysH include determining the required conditions for its crystallization mutating the active site (Figure 5) to confirm NTPase activity and determining if CasCysH plays a role in DNA and/or RNA modification.

CasCysH

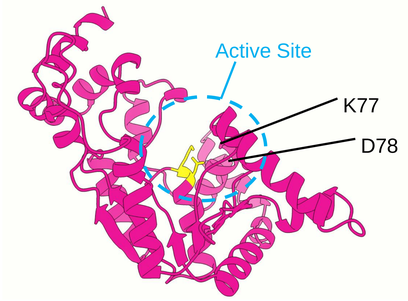


Figure 5: Alphafold 2 predicted structure of CasCysH. Putative active site residues (yellow) include a lysine (K77) and aspartic Acid (D78).

CasDinG

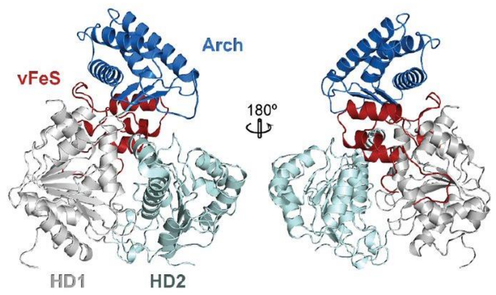


Figure 6: Model of CasDinG highlighting the Arch and vFeS accessory domains[1].

Future directions of this work with CasDinG include determining the role that the vFeS and arch domains play in Type IV-A immunity.

Acknowledgements

This work was supported by a National Institutes of Health National Institute of General Medical Sciences Maximizing Investigators' Research Award (R35GM138080) as well as a USU College of Science Minigrant award.

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