1. What is CRISPR?

2. What are the stages of CRISPR immunity?

CRISPR RNA processing by a Type IV Cas6 endoribonuclease

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USU Student Research Symposium
April 12, 2018

3. How diverse is CRISPR?

4. What is the function of the Type IV Cas6 protein?
1. What is CRISPR?

CRISPR is an adaptive bacterial immune system that defends bacteria against phage (viruses that infect bacteria).
2. What are the stages of CRISPR immunity?

Clustered
Regularly
Interspaced
Short
Palindromic
Repeats

CRISPR associated genes and proteins are called cas genes and Cas proteins

Adaptation
Remember the invader

Biogenesis
Build the defense machine

Interference
Destroy the invader

Cas gene
Repeat
Spacer
Cleavage site
3. How diverse is CRISPR?

<table>
<thead>
<tr>
<th>Interference complex:</th>
<th>Target:</th>
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I

II

III

IV

V

VI
Type I Cas6 proteins process the pre-crRNA into crRNAs.

- The Type I and Type IV CRISPR systems are similar &
- Type IV CRISPR systems have a cas6 gene

The Type IV Cas6 protein likely processes CRISPR pre-crRNAs into smaller RNAs.
4. What is the function of the Type IV Cas6 protein?

- The Type IV Cas6 processes pre-crRNAs into smaller RNAs
4. What is the function of the Type IV Cas6 protein?

- Structural comparison with Type I Cas6 proteins reveals the Type IV Cas6 active site.

*Pseudomonas aeruginosa* Cas6
From a *Type I* CRISPR system

*Mahella australiensis* Cas6
From a *Type IV* CRISPR system

*Thermus thermophilus* Cas6
From a *Type I* CRISPR system

Tyrosine 50 & Histidine 63
• The Type IV Cas6 cleaves the CRISPR repeat
• The amino acids His-63 and Tyr-50 are involved in the cleavage event
Significance

- The Type IV Cas6 protein does process the pre-crRNA during biogenesis.

Future Directions

- Where does the Type IV Cas6 cleave the repeat?
- Does Type IV pre-crRNA processing happen in vivo?
- Characterize more Type IV Cas proteins.
Acknowledgements

- Jackson lab
- Emily Warner – purified MaCas6 and mutants
- Ryan Jackson – solved the structure of MaCas6

References


Questions?