1. What is CRISPR?

2. What are the stages of CRISPR immunity?

CRISPR RNA processing by a Type IV Cas6 endoribonuclease

Hannah Taylor, Emily Warner, Valerie Crowley, Ryan Jackson
Utah State University, Department of Chemistry and Biochemistry

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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

Interference Complex

Diagram symbols:
- 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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<tr>
<td>I</td>
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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.

![Diagram showing the process of Cas6 converting pre-crRNAs into crRNAs over time.](image)
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
4. What is the function of the Type IV Cas6 protein?

- The Type IV Cas6 cleaves the CRISPR repeat
- The amino acids His-63 and Tyr-50 are involved in the cleavage event
• The Type IV Cas6 protein does process the pre-crRNA during biogenesis.

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?