Professor Ke’s Research Lab: The Study of the CRISPR-Cas System
Department of Molecular Biology and Genetics

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What is the CRISPR-Cas System?

- Clusters of Regularly Interspaced Palindromic Repeats
- Found in most bacteria and archaea
- Work with numerous adjacent Cas (CRISPR-associated) proteins as a defense system against foreign genetic elements
- Central to all CRISPR-Cas systems is a large ribonucleoprotein complex
  - Allows for the targeting and degradation of invasive genetic material
The Three Steps of the System

1.) Adaptation
Sample foreign genetic material placed as spacers in between palindromic repeats

2.) Expression
Transcription to form pre-CRISPR RNA
Processing of pre-CRISPR RNA (pre-crRNA) to mature form
Compilation of crRNA and Cas proteins

3.) Interference
Surveillance Complex
Target Degradation

John van der Oost, et. al. (2014). Unravelling the structural and mechanistic basis of CRISPR–Cas systems
Three Main Types of CRISPR-Cas Systems

• Type I System
  • Cascade utilizing an enzyme for locating, invading, and cleaving corresponding foreign DNA

• Type II System
  • Cas9 works as the helicase and the nuclease

• Type III System
  • Two types: Cmr and Csm
  • DNA interference and RNA interference

John van der Oost, et al. (2014). Unravelling the structural and mechanistic basis of CRISPR–Cas systems
Type I-E System

- Cascade
  - CRISPR Associated Complex for Antiviral Defense
  - Ribonucleoprotein surveillance complex
- Cas3
  - Helicase and nuclease
  - Recruited by Cascade

Ryan N. Jackson, et.al. (2015) Crystal structure of the CRISPR RNA–guided surveillance complex from Escherichia coli
Investigated Questions

Question

• How is Cas3 recruited by Cascade?

Prediction

• We hypothesized that conformational change of CasA leads to recruitment of Cas3.
  • Low-resolution electron microscopy structure of Cascade-Cas3 complex illustrates Cas3 adjacent to CasA
  • Comparison of apoform crystal structure versus dsDNA-bound Cascade illustrates conformational change of CasA
Mutants

• Mutant 1
  • Side-by-side double mutation on a surface loop
  • Switched two adjacent amino acids to alanine

• Mutant 2
  • Point mutation buried within conformational change interface
  • Switched one amino acid to alanine
Experimental Methods

Cloning: Site-Directed Mutagenesis → Transformation of Cells with Plasmid → Cell Growing and Protein Expression → Cell Harvesting and Cell Lysis → Ni-NTA Purification (Nickel Column) → Cas3 Cleavage Assay

CasA = Cse1
CasB = Cse2
CasC = Cas7
CasD = Cas5e
CasE = Cas6e
Ni-NTA Protein Purification (Nickel Column)

- **Nickel-Nitrilotriacetic Acid**
- Captures protein of interest
  - His-tag attached to the end of protein of interest
  - His-tag is made up of 6 histidines
  - His-tag adheres to column, isolating the protein

CasA = Cse1
CasB = Cse2
CasC = Cas7
CasD = Cas5e
CasE = Cas6e

*KPL, Kirkegaard & Perry Laboratories, Inc.*
SDS PAGE

- Sodium Dodecyl Sulfate Poly-Acrylamide Gel Electrophoresis
- Assesses the purity of the protein
- Checks for protein of interest
Wild type and Mutant 1 have identical results
  - Mutant was not in the same area as Cas3 association
In Mutant 2, there is no CasA

Recall:
- Mut 1 = Side-by-side double mutation, surface mutation
- Mut 2 = Point mutation, Buried mutation

CasA (55.9 kDa)
CasC (40 kDa)
CasD (26 kDa)
CasE (22 kDa)
CasB (19 kDa)

CasA = Cse1
CasB = Cse2
CasC = Cas7
CasD = Cas5e
CasE = Cas6e
Experimental Methods

1. Cloning: Site-Directed Mutagenesis
2. Transformation of Cells with Plasmid
3. Cell Growing and Protein Expression
4. Cell Harvesting and Cell Lysis
5. Ni-NTA Purification (Nickel Column)
6. Cas3 Cleavage Assay

Legend:
- CasA = Cse1
- CasB = Cse2
- CasC = Cas7
- CasD = Cas5e
- CasE = Cas6e
Cas3 Cleavage Assay

- Mix cascade with dsDNA substrate to form an R-loop
  - R-loop: dsDNA is recognized by Cascade. One strand binds to crRNA while the other one is displaced, making it susceptible to Cas3 cleavage
  - Cas3 is only capable of cutting single-stranded DNA
- For cleavage, must have:
  - Cascade/dsDNA R-loop - substrate
  - Cas3 – helicase/nuclease
  - ATP - energy
  - Catalytic metal – orients DNA phosphate for cleavage by activated water molecule (hydroxide ion)
Results

Rxn Conditions
10mM HEPES pH 7.5, 150 mM NaCl, 10 μM CoCl$_2$

1. Ladder
2. DNA alone
3. Cas3 + DNA + ATP
4. Cascade + DNA + ATP
5. Cascade + DNA + Cas3 + AMPPNP
6. Cascade + DNA + Cas3 + ATP
7. Cascade (Mut 1) + DNA + ATP
8. Cascade (Mut 1) + DNA + Cas3 + ATP
9. Cascade (Mut 2) + DNA + ATP
10. Cascade (Mut 2) + DNA + Cas3 + ATP
Rationalization for Lack of Cleavage Activity

- No CasA in mutant 2
- No cleavage occurred
- Failed to reject prediction
  - CasA must adhere to Cascade in order for cleavage to happen
Its Impact on the Future

• Cheaper relative to other DNA editing methods
  • Very versatile
  • Easy to make
  • Only materials you need = crRNA, targeting proteins

• Transcription/Gene expression
  • Catalytically-inactive CRISPR systems can be used to occupy promoter regions, ultimately altering downstream gene expression levels

• Holds the promise for clinically-relevant genome-editing applications
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