Module 2: Manipulating Metabolism

CRISPR: mechanism details

10/25/18



Identifying the cleavage target of Cas9

- Adaptive immune response that confers phage resistance
- Requires crRNA, tracrRNA, and Cas9

What is the target of the native system? Incoming viral DNA or host-transcribed viral mRNA?

DNA vs RNA debate

Many in researchers in phage community convinced RNA interference by CRISPR too inefficient given explosive replication of phage during infection



Data support that Cas9 cleaves DNA

1. Transformation of plasmid DNA blocked

2. Presence of self-splicing RNA sequence in DNA target abolished CRISPR activity

Native CRISPR system cleaves phage DNA

 Adaptive immune response encoded by CRISPR loci and Cas genes



- Mechanism involves three stages:
 - Adaptation
 - Expression
 - Interference

CRISPR system: adaptation



Phage DNA recognized and fragmented by restriction enzyme system, then 'spacers' incorporated into bacterial genome

CRISPR system: expression



Cas9 and Rnase III involved in processing precrRNA, then Cas9 forms complex with crRNA and tracrRNA

CRISPR system: interference



Cas9 / tracrRNA / crRNA bind invading phage DNA and cleave at target sequence that is complementary to 'spacer' sequence

Proteins involved in CRISPR systems



Koonin and Makarova. (2013) RNA Biology. 10:679-686.

Engineered CRISPRi system

• Modifications to crRNA / tracrRNA complex?

• Modifications to Cas9?

CRISPRi system: (s)gRNA

 (s)gRNA molecule is a target sequence and tracrRNA fused by a linker loop such that a single transcript used to direct Cas9 cleavage



sgRNA able to target Cas9 cleavage



CRISPRi system: dCas9

 dCas9 protein contains mutated residues D10A and H840A that render it catalytically inactive and unable to cleave DNA, but still able to bind DNA

HNH and RuvC endonuclease domains

- RuvC
 - Endonuclease that resolves Holliday structure, intermediate structure in which dsDNA molecule is linked by single-stranded crossover
- HNH
 - Found in homing endonucleases, restriction endonucleases, transposases

Cleavage requires HNH and RuvC domains

Jinek et al. (2012) *Science*. 337:816-820.

HNH and RuvC domains target specific DNA strands

Jinek et al. (2012) Science. 337:816-820.

Schematic of Cas9 DNA cleavage

- RuvC domain (D10A) cleaves non-coding strand
- HNH domain (H840A) cleaves coding strand

 Result in blunt end cut 3 bp from PAM site

Role of PAM in CRISPR system

Crystal structure of Cas9 / sgRNA

In the laboratory...

- Mini-prep pgRNA_target clones
- Transform CRISPRi system into competent MG1655
 - pdCas9
 - pgRNA_target
- Send pgRNA_target clones for sequencing

