Investigating the Role of Single-Stranded DNase Activity By the CRISPR-Cas12a System

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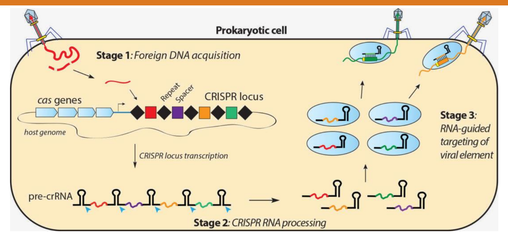
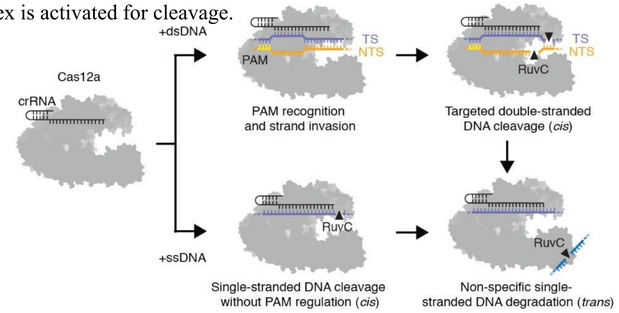
Abstract: CRISPR-Cas is an RNA-guided adaptive immune system found in many bacteria and archaea. Recently, studies have shown that a CRISPR-Cas system, known as Cas12a, cleaves single-stranded DNA nonspecifically; however, the biological role of this activity is not well understood. Cas12a's single-stranded DNA cleavage activity is similar to that of a different CRISPR-Cas system, known as CRISPR-Csm, which specifically targets transcriptionally active DNA. The similarities between these two systems has stemmed interesting questions regarding Cas12a's cleaving potential. The main goal of this study is to test whether Cas12a can cut transcriptionally active DNA using *in vitro* assays. We will also compare the DNA cleavage activities of the two CRISPR-Cas systems, and test if Cas12a targeting *in vivo* causes cell toxicity. This research could reveal unexpected connections between different CRISPR-Cas systems and lead to new ways of harnessing CRISPR-Cas systems with single-stranded DNA cutting activity for genome engineering.

CRISPR-Cas System and Cas12a



[1]

CRISPR-Cas systems are adaptive immune systems of bacteria and archaea. After initial exposure, the phage's DNA is stored in the CRISPR locus and transcribed. The mature CRISPR RNA is then assembled with a CRISPR-Cas protein and together the protein complex is activated for cleavage.

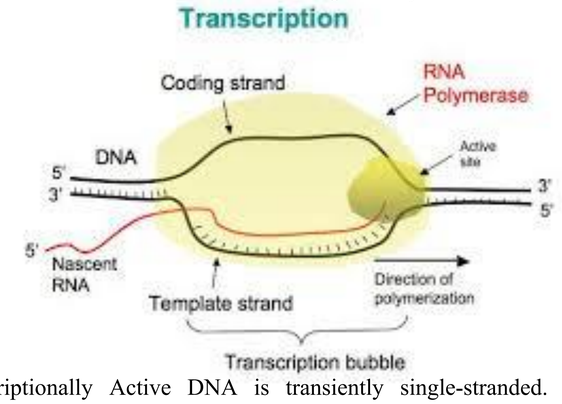


[2]

CRISPR-Cas12a is activated for nonspecific single-stranded DNA (ssDNA) cleavage two ways: Complementary double-stranded DNA (dsDNA) binding and cleavage, or complementary ssDNA binding and cleavage.

Objectives

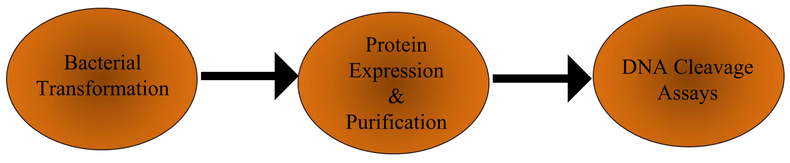
- Test whether LbCas12a cuts transcriptionally active DNA.



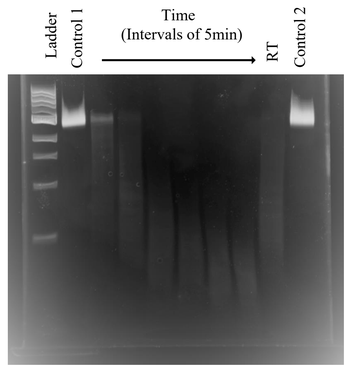
[3]

Transcriptionally Active DNA is transiently single-stranded. Therefore, we hypothesize CRSIPR-Cas12a can cleave transcriptionally active DNA in trans.

Methods



DNA Cleavage Assays



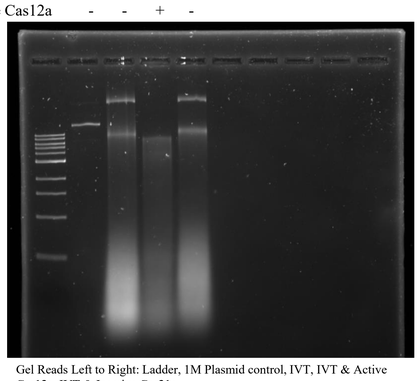
Control 1: M13 & crRNA & Cas12a. Not Incubated at 37 °C.
Control 2: M13 & crRNA & Cas12a. Incubated at 37 °C for 30 Minutes.
RT: Room Temperature.

M13 Cleavage Assay

- LbCas12a active.
- Rapid degradation of M13.
- Suggests warmer temperatures increases LbCas12a's cleavage activity.

In Vitro Transcription Cleavage Assays

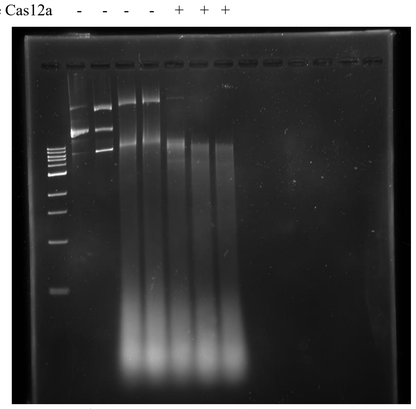
Active Cas12a - - + -



Gel Reads Left to Right: Ladder, 1M Plasmid control, IVT, IVT & Active Cas12a, IVT & Inactive Cas21a.

- DNA band is missing.
- Decreased RNA production.

Active Cas12a - - - - + + +



Gel Reads Left to Right: Ladder, 1M Plasmid control, Inactive IVT & Active Cas12a, IVT, IVT & Inactive Cas12a, IVT & Active Cas12a in Increasing One Hour Time Intervals (1hr-3hr).

- Top two DNA bands disappeared.
- Decreased smearing in active Cas12a samples.

Data Analysis

- LbCas12a does have a role in IVT Cleavage Assays, but it's exact function is to be determined.
- Possible contamination of sample and/or T7 or other protein binding to DNA in IVT Assays could explain shifts in plasmid location.

Conclusion

- LbCas12a verified active
- Warmer temperatures (i.e 37 °C) potentially increases LbCas12a's cleavage ability.
- Unclear whether CRISPR-Cas12a can cut transcriptionally active DNA, further investigation is needed.

Further Work

- Visualize IVT Cleavage Assay on an SDS-Page and radiolabel DNA for better resolution and clarity.
- Test IVT Cleavage Assay with E.Coli polymerase.
- Compare cleavage kinetics of CRISPR-Csm to CRISPR-Cas12a.
- Test whether Cas12a cleavage *in vivo* causes cell toxicity.

References

[1] http://doudnalab.org/research_areas/crispr-systems/
[2] Chen JS, Ma E, Harrington LB, Da Costa M, Tian X, Palefsky JM, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science. 2018;eaar6245.
[3] http://www.onlinebiologynotes.com/transcription-in-prokaryotes/

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