MECHANICS OF R-LOOP FORMATION BY CASCADE STUDIED WITH MAGNETIC TWEEZERS

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Bacteria and archaea use CRISPR (clustered regularly interspaced short palindromic repeats) to defend against bacteriophages by targeting foreign nucleic acid for degradation. In type I CRISPR systems, the crRNA guide is bound and directed by the Cascade effector complex [1]. The Cascade complex recognizes the PAM sequence on the target DNA and identifies the target by formation of the R-loop structure – a three-stranded nucleic acid structure, composed of a DNA-RNA hybrid and the displaced non-target single-stranded DNA. The formation of a stable R-loop is a crucial step in the CRISPR mechanism because it permits recruitment of the nuclease-helicase Cas3, which initiates degradation of the target DNA [Fig. 1], [2].

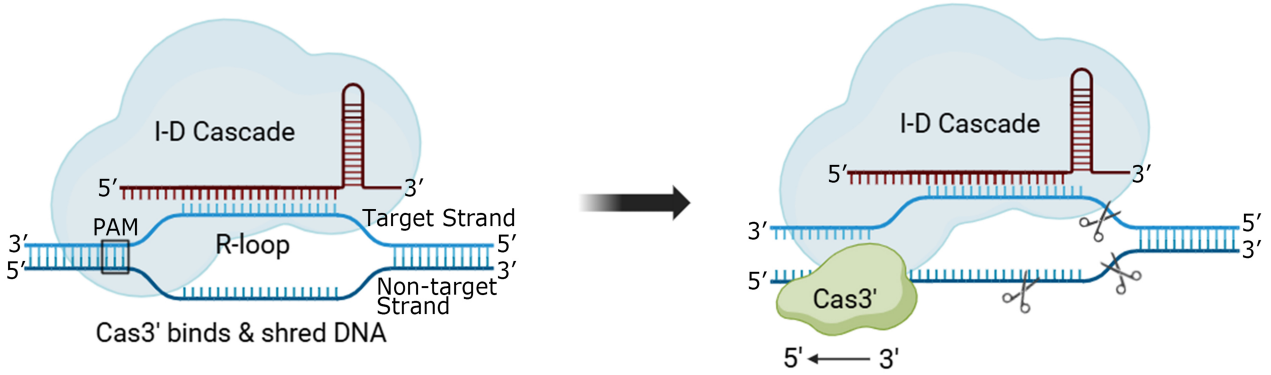

Fig. 1. The Type I-D Cascade binds its target and forms an R-loop, creating a platform for Cas3' to unwind and shred the DNA.

In this study, we investigate the mechanics of the R-loop formation in real-time at the single-molecule level using magnetic tweezers. This technology allows observation of dynamic conformational changes and the timing of individual R-loop-forming molecular events. By changing the force on a DNA-bound paramagnetic bead or introducing torque, we probe how R-loop stability depends on mechanical tension and torque. We compare Cascade complexes from different species to better understand the initiation and regulation of R-loops.

[1] Y. Zheng et al., "Endogenous Type I CRISPR-CAS: From foreign DNA defense to prokaryotic engineering," Frontiers in Bioengineering and Biotechnology, vol. 8, p. 62, Mar. 2020, doi: 10.3389/fbioe.2020.00062.
[2] K. Yoshimi et al., "Dynamic mechanisms of CRISPR interference by Escherichia coli CRISPR-Cas3," Nature Communications, vol. 13, no. 1, p. 4917, Aug. 2022, doi: 10.1038/s41467-022-32618-0.