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Poster #56: Stepwise R-Loop Unwinding and Cas3-Mediated Target Degradation in a Multiprotein CRISPR–Cas System

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crRNA-guided effector complexes (ECs) of CRISPR–Cas systems mediate the cleavage or silencing of invading phage genetic material. Class 1 CRISPR–Cas systems, which rely on multiprotein effector assemblies, are the most abundant CRISPR systems in nature [1] and have been widely adapted for biotechnological applications, including genome engineering and transcriptional silencing [2]. In type I CRISPR systems, the crRNA-guided Cascade complex recruits the Cas3 nuclease–helicase, which drives processive and unidirectional (3′⟶5′) degradation of target DNA [3].

Here, we investigate the molecular mechanism of CRISPR interference by resolving four Cryo-EM structures capturing intermediate steps of target recognition and stepwise R-loop propagation. Together with complementary biochemical analysis, these structural snapshots reveal how R-loop formation induces the conformational rearrangements required to expose the Cas3-binding interface and initiate target degradation. Mutational analyses identify key Cascade–Cas3 interactions that are essential for interference, while other contacts appear to be functionally dispensable. Furthermore, we examine the contribution of these interactions to primed adaptation and show that this process requires less stringent Cascade–Cas3 coordination than target interference.

Reference #1

Makarova, K. S., et al. (2025). ``An updated evolutionary classification of CRISPR-Cas systems including

Reference #2

Liu, T. Y., Doudna, J. A. (2020). Chemistry of Class 1 CRISPR-Cas effectors: Binding, editing, and regulat

Reference #3

Šinkūnas, T., et al. (2013). ``In vitro reconstitution of Cascade-mediated CRISPR immunity in Streptococcu

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