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MECHANISM OF CRISPR-CAS3 HELICASE USING MAGNETIC TWEEZERS

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CRISPR-Cas provides RNA-guided adaptive immunity against invading genetic elements. CRISPR systems consist of multiple Cas proteins responsible for CRISPR-dependent cell immunity mechanisms. The effector complex in CRISPR I-E consists of Cascade and Cas3. Cascade is responsible for foreign DNA targeting. Meanwhile, Cas3, which possesses helicase and nuclease activity, is a key system protein, necessary for crRNA-guided interference of virus proliferation. Although single-component Class 2 CRISPR systems, such as type II Cas9 are widely used for genome editing, the research on multi-component Class 1 proteins of the same system has been less developed. Components of the I-E CRISPR system have already been used as a genome-editing tool to generate big deletions. However, the detailed mechanism by which Cas3 achieves its function is not well understood. This study aims to elucidate the mechanism of Cas3 unwinding. We are using single-molecule force microscopy, namely, magnetic tweezers, to probe the mechanical aspects of Cas3 unwinding activity. Greater knowledge of the Cas3 mechanism of action would improve the application of Cas3 as a tool for genome editing.