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STRUCTURAL BIOLOGY

Cascade keeps its targets in the loop

> mechanistic insights into PAM-dependent R-loop formation, Cas3 recruitment and substrate handover

In type I CRISPR–Cas systems, foreign DNA is detected by the CRISPR-associated complex for antiviral defence (Cascade). This surveillance complex contains Cas proteins and CRISPR RNA (crRNA), which recognize DNA target sequences (protospacers) that are flanked by an optimal protospacer-adjacent motif (PAM). During the target-searching process, crRNA binds to the complementary target DNA strand to form a seed bubble, whereas the non-targeting strand is displaced (R-loop formation). Subsequently, the nuclease–helicase enzyme Cas3 is recruited to degrade the non-target and target DNA strands.

This study presents cryo-electron microscopy structures of the type I-E Cascade from *Thermobifida fusca*, which forms a seed sequence bubble and a full R-loop structure, at 3.8 Å and 3.3 Å resolution, respectively, thus capturing the early events in R-loop formation. These structural snapshots revealed that PAM recognition is coupled to the bending of the target DNA and spontaneous DNA unwinding, which leads to the establishment of a seed bubble as the DNA–crRNA heteroduplex forms. Further unwinding of the entire protospacer leads to the completion of R-loop formation, which triggers a conformational change in Cascade. Moreover, the degradation factor Cas3 was shown to specifically bind to Cascade once R-loop formation was completed, which suggests a regulatory mechanism to prevent premature recruitment of Cas3 until the entire protospacer region has been sequence validated by the crRNA guide. Finally, Cascade was shown to create a bulge in the non-target DNA strand that facilitates the exchange of the non-target DNA strand from Cascade to Cas3 for degradation. Collectively, the structures provide mechanistic insights into PAM-dependent R-loop formation, Cas3 recruitment and substrate handover.

Andrea Du Toit

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