INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

Archaea and Bacteria defend themselves from the assault of phages and plasmids by employing a genetically encoded RNA mediated adaptive immunity referred to as the CRISPR-Cas system. The CRISPR-Cas systems are highly diverse and classified into several subtypes. Unlike the Cascade/I-E (from type I-E system), which is composed of five subunits, the Cascade/I-C (from type I-C system) is made up of four subunits lacking the CRISPR RNA processing enzyme Cas6, whose role is assumed by Cas5. How these differences in the composition and organization of Cascade subunits in type I-C influence the Cas3/I-C binding and its target cleavage mechanism is poorly understood. Here, we show that Cas3/I-C is intrinsically a single-strand specific promiscuous nuclease. Apart from the helicase domain, a constellation of highly conserved residues—unique to type I-C—located in the uncharacterized C-terminal domain appears to influence the nuclease activity. Recruited by Cascade/I-C, the HD nuclease of Cas3/I-C nicks the single-stranded region of the non-target strand and positions the helicase motor. Powered by ATP, the helicase motor reels in the target DNA until it encounters the roadblock en route, stimulating the HD nuclease. Remarkably, we have shown that Cas3/I-C supplants Cas3/I-E for CRISPR interference in type I-E in vivo, suggesting that the target cleavage mechanism is evolutionarily conserved between type I-C and type I-E despite the architectural difference exhibited by Cascade/I-C and Cascade/I-E. On the contrary, when the target DNA is mutated, this evades target DNA recognition and cleavage. And this turns on an inflammatory response leading to priming spacer acquisition or primed adaptation. Here, we have shown that in type I-C, functional Cas3/I-C enhances the frequency of spacer acquisition by functioning in cooperation with the Cascade and the Adaptation complex. A three-nucleotide protospacer adjacent motif (PAM) sequence seems to play a crucial role in diverting interference complex to the spacer acquisition process. Remarkably, the Cascade, which guides the Adaptation complex towards the priming site, does not appear to physically interact with the Adaptation complex. Additionally, we have shown that Cas3/I-C CTD, which is crucial for interference, is equally obligatory during primed adaptation. Overall, the thesis uncovers the crucial molecular events that occur during the target DNA cleavage and the cross-talk between the interference and the adaptation stages in the type I-C CRISPR-Cas system.