INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

CRISPR-Cas system represents a genetically encoded, small RNA guided adaptive defence mechanism that utilises the phage-derived nucleic acid as an immunological memory to guide the destruction of foreign DNA/RNA by Cas effector nuclease. The small guide RNA that is referred as CRISPR RNA (crRNA) is synthesised as Pre-CRISPR transcript, which further undergoes processing by a specific Cas nuclease to become the mature crRNA. The ongoing evolutionary arms race between phages and bacteria drives the emergence of new variants of CRISPR-Cas system. These variants exhibit unusual variation in the composition and architecture of Cas proteins. To understand how these structural differences, translate into functional adaptations, we probed the mechanism of CRISPR RNA maturation in a newly discovered type I-G. In *Bifidobacterium animalis* that encodes type I-G, we identified putative promoter sequences on both strands of the CRISPR array, suggesting that CRISPR is transcribed from both orientations. The *cas* operon, among other *cas* genes, encodes three Cas proteins, viz, Csb1, Csb2 and Csb3 that assemble with crRNA to form the ribonucleoprotein surveillance complex referred as Cascade complex. Remarkably, Csb2 is a metal independent endonuclease that cleaves site-specifically within the CRISPR repeat RNA that is synthesized from both orientations. The catalytic activity of Csb2 resides within the C-terminal region that is homologous to Cas6, while the N-terminal Cas5 is inert. Csb2 processes the pre-CRISPR transcript as a stand-alone enzyme as well as a subunit of the Cascade complex. Surprisingly, we discovered that Csb1 - which is homologous to Cas7 that is catalytically inert in other type I systems- also shows RNase activity that is functionally analogous to Csb2 in processing the CRISPR repeat RNA. crRNA produced from both orientations elicits robust CRISPR interference *in vivo*, suggesting that Csb1/Csb2 mediated RNA processing is effective for both orientations of pre-CRISPR transcript. Our work suggests that the presence of disparate dual nucleases in the Cascade complex with functional convergence enhances the efficiency of CRISPR-based immunity in type I-G.