INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

CRISPR-Cas based adaptive immunity protects the genome of bacteria and archaea from the attack of foreign genetic elements such as bacteriophages and plasmids. A specialized region on the genome termed CRISPR locus is the activity center for this small RNA-based immune response. CRISPR locus comprises numerous short repeats interspaced by equal-sized spacers and an AT-rich leader. When the bacteriophage attacks the bacterium, the bacteriophage DNA is broken down into small fragments (prespacers), which are then integrated into the CRISPR array as spacers. These bacteriophage derived spacers act as DNA-based infection memory – akin to protein-based antibodies in higher organisms like humans – to counter recurrent infection. The process of generating infection memory is called CRISPR adaptation. The spacers derived from the invader are precisely sized and always integrated at the leader-proximal repeat in the CRISPR array. In this work, the molecular mechanism of CRISPR adaptation in type I-E CRISPR-Cas system is addressed using model bacterium *Escherichia coli*. In the type I-E CRISPR-Cas system, two universally conserved proteins Cas1 and Cas2, form an integrase complex and catalyze the CRISPR adaptation. Exonucleases trim the Cas1-2 bound DNA to produce a 33 bp prespacer that holds the infection memory. A nucleoid protein called IHF cooperates with the Cas1-2-prespacer integrase complex to store the infection memory. IHF interacts with the CRISPR leader and restructures this region to generate a cognate binding site for Cas1-2-prespacer at the leader proximal repeat. This process allows the integration of 33 bp DNA fragment in a sequential order into the CRISPR array. These intricate mechanistic details of infection memory generation demystify how bacteria use the CRISPR-Cas system to recognize and counter the recurrent invasion of bacteriophages.