INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: **INVESTIGATIONS ON THE MATURATION OF CRISPR RNA IN TYPE I-C CRISPR-CAS SYSTEM**

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Thesis Submitted to the Department/ Center	:	**Biosciences and Bioengineering**
Date of completion of Thesis Viva-Voce Exam	:	**12th April 2017**
Key words for description of Thesis Work	:	CRISPR, Cas, Cascade, RNAi, DNase, RNase, Prokaryotes, adaptive immunity, guide RNA, ribonucleoprotein complex, co-transcriptional processing

SHORT ABSTRACT

Adaptive immunity is a recent discovery in prokaryotes, which was previously thought to be present only in vertebrates. The system, referred as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) in association with CRISPR-associated (Cas) proteins provides adaptable and heritable immunity against mobile genetic elements in prokaryotes. It adapts by integrating short sequences from the invading nucleic acid into the host CRISPR locus to generate immunological memory – akin to antibodies in higher organisms which adapt according to specific antigen. The integrated foreign DNA is then transcribed and processed to form mature CRISPR RNA (crRNA), which along with Cas proteins forms the ribonucleoprotein (RNP) surveillance complex to combat subsequent infections. The work presented in the thesis uncovers the unique features of type I-C CRISPR-Cas system, which utilizes only three Cas proteins *viz.*, Cas5d, Csd1 and Csd2 to form the RNP complex. Unlike other type I CRISPR systems that employ Cas6 or its homologs for metal independent CRISPR RNA maturation, the type I-C that lacks this, deputes Cas5d and Csd1 to process the pre-crRNA. The homogeneity of crRNA population seems to be achieved by coupling the transcription and processing of CRISPR array. A startling revelation is the identification of promiscuous DNase activity of Cas5d and Csd1 that is selectively promoted in the presence of divalent metals. Though Csd1 is biased towards double stranded DNA, Cas5d is proficient against all forms of DNA. This may enable them to be co-opted in adaptation and interference stages of CRISPR immunity, wherein direct interaction with the foreign DNA is involved. Remarkably, the same active site that renders RNA hydrolysis may be tuned by metal to act on DNA substrates too. The key to the activity switching is the affinity modulation towards the metal cofactor. In the absence of DNA, Cas5d shows weak affinity towards metal (35.79 mM), allowing it to function as RNase, while the affinity enhancement in presence of DNA (1.33 µM) renders it to be a DNase. Further, Cas5d and Csd1 assemble with inert Csd2 to form the type I-C surveillance complex, which possesses RNase activity but no apparent DNase activity. This parallel processing of the crRNA seems to be an evolutionary adaptation for eliciting a rapid immune response. Thus, the work highlights the moonlighting function of type I-C molecular machinery that appears to have potentiated the CRISPR immunity against genome invaders.