INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Programme of Study	:	**Ph.D.**

Thesis Title: **Transcriptome Profiling of UPF3B-KO Cells and Its Implication in Alternative Splicing-Coupled Nonsense-Mediated Decay**

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SHORT ABSTRACT

Nonsense-mediated mRNA decay determines the fate of aberrant transcripts identified by the pioneer round of translation. UPF3B, in association with UPF2 and UPF1, assembles over transcripts marked for decay. Phosphorylation of UPF1 recruits SMG proteins, ultimately leading to the degradation of faulty transcripts. The assembly of distinct protein complexes defines various routes of NMD pathways, with UPF3B playing a role in multiple branches. However, the influence of UPF3B on the transcriptome regulation and its implications for NMD activity remain poorly defined. To investigate the effect of UPF3B deficiency, two UPF3B-KO clones were generated using CRISPR/Cas9 technology. Intriguingly, RNA-Sequencing of UPF3B-KO cells and differential gene expression analysis revealed elevated levels of GADD45 and selenoprotein transcripts, which are potential physiological NMD targets. Moreover, the complete loss of UPF3B resulted in the upregulation of canonical NMD substrates and an NMD reporter control, PTC39 β-globin, demonstrating impaired NMD activity. Impairment in NMD activity also triggers the autoregulatory feedback mechanism of NMD factors. Remarkably, gene expression profiling at transcript-level identified 165 NMD transcripts and 153 novel PTC+ transcripts in UPF3B-KO clones, highlighting their regulation by UPF3B-mediated NMD. Subsequently, isoform switching analysis demonstrated increased usage of NMD-sensitive splice isoforms, such as SAT1 and HNRNPA2B1, underscoring the splicing-associated regulatory role of UPF3B. These findings suggest that UPF3B is associated with alternative splicing coupled with NMD (AS-NMD) and functions in restraining unproductive splicing events. Altogether, results from this study reveal novel NMD substrates of the UPF3B-dependent surveillance pathway, indicating that UPF3B is required for NMD function in a context-dependent manner.