INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: TO GAIN INSIGHTS INTO THE FUNCTION OF YEAST DNM1 IN MITOCHONDRIAL DYNAMICS

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SHORT ABSTRACT

Mitochondria, vital hubs of cellular metabolism, continuously modulate their shape and number through fission and fusion. The central mediator of mitochondrial fission is the GTPase Dnm1 in yeast, and its homolog DRP1 in humans. Dnm1 comprises four domains - an N-terminal GTP-binding domain, a middle domain, a variable B-insert domain, and a C-terminal GTPase effector domain. Dnm1 undergoes assembly/disassembly cycles driven by GTP binding and hydrolysis to facilitate fission. While substantial progress has been made in understanding the domain architecture, function, and interacting partners of Dnm1, critical aspects, including the regulation of oligomeric forms, their spatio-temporal distribution, and the impact of post-translational modifications (PTMs), remain unclear. Moreover, disruptions in the delicate balance between mitochondrial function and dynamics are associated with various human diseases, with specific mutations in DRP1 linked to pathological conditions. Despite previous studies on the effects of these mutations on mitochondrial morphology, their impact on protein localization, distribution, function, and structure remains unexplored. This study aimed to investigate specific residues in Dnm1 that may undergo modifications or are mutated in disease conditions, examining their impact on the protein's structure, localization, and function. To achieve this, functional FL-Dnm1-GFP and Dnm1-HisHA fusion proteins were constructed, for *in vivo* and *in vitro* assessment, respectively. Five putative Dnm1 phosphorylation sites were selected for mutagenesis based on stringent conservation criteria. Interestingly, mutating S624, analogous to the reported regulatory DRP1 S616 site, did not affect mitochondrial morphology in yeast. However, mutating T62 and S277 in G2 and G5 motifs of the GTPase domain yielded non-functional proteins despite differences in their localization and dynamics. Structurally, T62A/D formed atypical large puncta, while S277A/D resembled WT Dnm1. Further computational analyses and molecular dynamics simulations provided insights into conformational changes and altered atomistic motion, particularly highlighting the dominant-negative impact of S277 mutation without altering protein localization. Furthermore, the study extended the investigation to mimic four disease-causing DRP1 mutations in Dnm1, uncovering diverse functional outcomes. For instance, the A430D mutation led to a complete loss of Dnm1 function, disrupting typical punctate phenotypes and presence of diffused cytosolic fluorescence, indicating defective oligomerization. Simulations revealed the mutation induced major conformational and dynamics changes in a helix region. In contrast, the G397D mutation resulted in fewer, larger, and less dynamic puncta, likely due to change in orientation of a loop surrounding the mutated residue. Thus, investigating each mutation in detail is crucial for gaining insights into their respective roles in disease-associated alterations of mitochondrial dynamics. In conclusion, this research provides novel insights into the molecular basis of Dnm1 function and regulation, contributing to a deeper understanding of conserved mitochondrial fission processes. Additionally, it paves the way for the development of targeted therapies for several neurological diseases where mitochondrial fission-fusion regulation is impaired.