

DRP1 MIDDLE DOMAIN INTERACTIONS AND MITOCHONDRIAL MORPHOLOGY

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Mitochondria are critical cellular organelles that generate ATP, regulate redox and metabolism, maintain calcium homeostasis, and oxidize fatty acids. Mitochondria dynamically fuse and divide within cells, and the balance of fusion and fission helps maintain function and morphology. Mutations in proteins involved in these processes cause neurological disorders such as Charcot-Marie-Tooth neuropathy and optic atrophy type 1. Alterations in mitochondrial fusion and fission are involved in apoptosis, chemotaxis, and aging. The dynamin-related protein 1 (Drp1) is a conserved, GTPase responsible for mitochondria fission, likely by forming large multimeric spirals at mitochondrial fission sites. The Drp1 protein harbors four motifs: GTP-binding, middle, insert B, and GTPase effector (GED). The intra-molecular interaction between the N-terminal GTPase domain and C-terminal GED is critical for Drp1 function. The middle domain is important for assembly of Drp1 oligomers into higher-order structures. Recently, a mutation at residue 395 (A395D) within the Drp1 middle domain was reported in a neonate with microcephaly, abnormal brain development, optic atrophy, and lactic acidemia; she died at 37 days. The mitochondria in patient fibroblasts were markedly elongated, but the molecular mechanisms were not addressed. We investigated effects of this A395D mutation and two other middle domain mutations (G350D, G363D) on Drp1 function. Using yeast two hybrid tests, chemical cross-linking, and gel exclusion FPLC, we found all these mutations compromised higher-order assembly, but not oligomer formation. Intramolecular interactions were also impaired, and GTPase activity was decreased. Mutant proteins impaired Drp1 localization to mitochondria, indicating that higher-order assembly is important step for mitochondrial localization. Finally, overexpression of these mutants inhibited mitochondria fission, with accumulation of aberrantly elongated mitochondria. Thus, Drp1 A395D lethal defect likely resulted from impaired higher-order assembly of Drp1 at mitochondria, leading to decreased fission, markedly elongated mitochondria, and impaired cellular distribution of mitochondria.