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Structural Studies that Define Regulatory Interactions within the Mitochondrial Fission Machinery

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Mitochondrial fission is essential for distributing cellular energy throughout cells and for isolating damaged regions of the organelle that are targeted for degradation [1]. This multistep process is initiated by the enhanced recruitment and oligomerization of dynamin-related protein 1 (Drp1) at the surface of mitochondria (**Fig. 1**). In fact, Drp1 is essential for inducing mitochondrial division in mammalian cells [2, 3], and homologous proteins are found in all eukaryotes. Drp1 localization within cells is largely cytosolic, but it assembles on mitochondria at sites of ensuing fission.

The molecular mechanism by which Drp1 is recruited from the cytosol to the mitochondrial surface is largely debated. In part, mitochondrial interactions are mediated through integral membrane receptors. Several Drp1-partner proteins have been discovered, but it is unclear whether their functions are unique or redundant. Additionally, interactions with specific lipids at the outer mitochondrial membrane (OMM) coordinate assembly of functional complexes that mediate membrane scission [4]. The presence of a mitochondrial specific lipid, cardiolipin, in membrane templates stimulates Drp1 activity by promoting self-assembly into larger helical polymers that we aim to study using electron microscopy (EM) and complementary methods.

To achieve this goal, our lab has examined Drp1 assemblies on various lipid templates using structural and functional studies [4-6]. There are similarities with other mechanochemical dynamins [7-9], but we continue to find novel attributes within the mammalian mitochondrial fission machinery. Specifically, Drp1 interactions with distinct lipids reveal structural differences that have the potential to augment Drp1 activity. This relationship with lipid is coincident with partner protein binding, which also regulates the assembly and subsequent GTPase activity of Drp1 [10-12]. Therefore, Drp1 interactions with partner proteins will be studied in a membrane-proximal environment using novel lipid templates [13]. In this way, the separate (and combined) effects of lipid and protein interactions are reconstituted to mimic conserved interactions at the surface of the OMM that drive mitochondrial fission.

Using cryo-EM, our studies will identify key molecular interactions that promote assembly of the mitochondrial fission machinery. Moreover, three-dimensional structures of human Drp1 will be explored in distinct states mediated by specific lipid, protein and nucleotide interactions. Conformational changes in the presence of different lipid cofactors will identify their role in promoting membrane scission. Additionally, we will characterize connections between Drp1 and its partner proteins to identify structural changes in the Drp1 oligomer that regulate functional progression, or membrane constriction, at the surface of mitochondria. Given the variety of Drp1 partners, several modes of regulation are plausible. Collectively, these studies will reveal the relationship between mitochondrial fission and factors that regulate this fundamental process [14].

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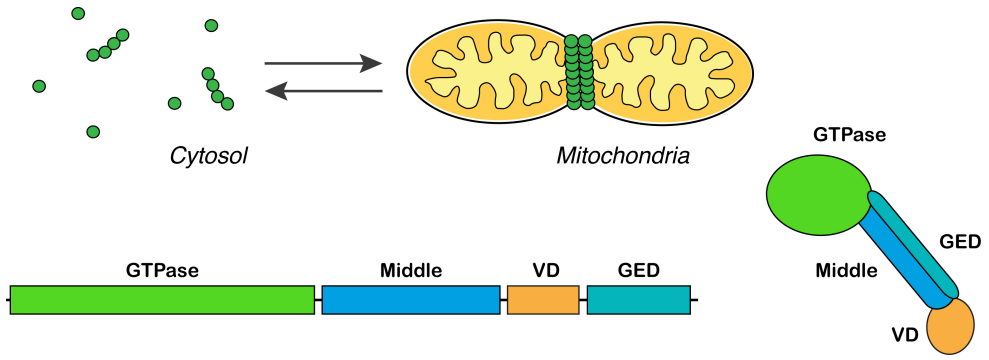

Figure 1. Dynamin-related protein 1 (Drp1) is the key mediator of mitochondrial fission. It is recruited to the surface of mitochondria through protein and lipid interactions that are only beginning to be elucidated. Self-assembly at the surface of mitochondria is mediated through middle and GTPase effector domain (GED) interactions, and GTPase activity is essential for imparting contractile force. More recently, the variable domain (VD) of Drp1 has been identified as a regulatory domain.

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