Mitochondrial fusion: Reaching the end of mitofusin's tether

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In this issue, Qi et al. (2016. *J. Cell Biol.* https://doi.org/10.1083/jcb.201609019) provide structural insights into the mechanisms of mitochondrial outer membrane fusion by investigating the structure of mitofusin 1 (MFN1). This work proposes a new model to explain the important and elusive process of MFN-mediated mitochondrial fusion.

Mitochondria undergo constant fission and fusion events, resulting in a highly dynamic and reticular network specialized for cellular function. The mitochondrial outer membrane proteins mitofusin 1 and mitofusin 2 (MFN1 and MFN2) were identified as membrane-bound, dynamin-related proteins essential for tethering adjacent mitochondria and executing outer membrane fusion (Hales and Fuller, 1997; Santel and Fuller, 2001). Inner membrane fusion is subsequently achieved by the action of a separate dynamin-related protein, OPA1 (Song et al., 2007). Fusion is important for ensuring a homogenous network of mitochondria within the cell, whereas hyperfusion of mitochondria acts as a transient stress response to protect cells from undergoing cell death. Mutations in genes encoding the fusion mediators MFN2 and OPA1 lead to neurological disease, including Charcot-Marie-Tooth type 2A (CMT2A) neuropathy and autosomal-dominant optic atrophy, respectively (Escobar-Henriques and Anton, 2013).

MFN1 and 2 have the same topology with two transmembrane regions with N- and C-terminal domains facing the cytosol. The N-terminal domain contains a GTPase domain followed by a heptad repeat (HR1), whereas the C-terminal domain contains a second HR (HR2). The current model of MFN-dependent mitochondrial fusion suggests that cis-dimerization of MFNs occurs on the mitochondrial surface followed by trans-association on adjacent mitochondrial membranes (Ishihara et al., 2004; Koshiba et al., 2004). The first insights into how MFNs tether membranes came from the structure of the C-terminal region of MFN1 encompassing the HR2 domain (Koshiba et al., 2004). It was found that this region dimerizes with another HR2 domain to form a long antiparallel coiled coil. This led to the conclusion that the HR2 domain is required for trans-association, producing a mitochondrial tether that precedes membrane fusion. The mechanism by which the membranes fuse is not clear but is dependent on the hydrolysis of GTP via the MFN GTPase domain. In a study reported in this issue, Qi et al. obtain crystal structures for part of MFN1, thereby providing new insights into the structural basis of MFN function.

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Qi et al. (2016) generated a variant of MFN1 that they termed mini-MFN1, which consists of the N-terminal GTPase domain of MFN1 linked to the second half of the HR2 domain in the C-terminal tail (Fig. 1 A). In contrast to other constructs tested, mini-MFN1 was recombinantly expressed and purified as a soluble protein. The subsequent 2.6-Å crystal structure of mini-MFN1 was solved in the presence of GDP and was found to consist of a typical GTPase domain and a four-helix bundle termed helix bundle 1. This four-helix bundle is composed of two helices from the GTPase N-terminal extension, a helix extending from the end of the GTPase domain, and surprisingly, the helix from the second half of the C-terminal HR2 domain. Is this structure relevant to the structure of the complete MFN1? The most likely answer is yes. This is because the structure of mini-MFN1 closely resembles the structural organization of the bacterial dynamin-like protein (BDLP; Low et al., 2009). Helix bundle 1 in mini-MFN1 closely resembles that of the neck domain of BDLP in both the nucleotide-bound and Apo forms. Most relevant to the structure of mini-MFN1, of the four helices making up the neck domain of BDLP, one comes from the last C-terminal helix of the entire protein, which loops up from the membrane-binding region.

The authors confirmed that mini-MFN1 behaved in a similar way to full-length MFN1 by finding it dimerized in the presence of GTP and not GDP or in the absence of any nucleotide, as previously shown (Ishihara et al., 2004). A GTP-binding defective mutant (K88A) of mini-MFN1 also did not dimerize. By attaching mini-MFN1 to the transmembrane region of *Drosophila melanogaster* atlastin (a related member of the dynamin family involved in the fusion of ER membranes, atlastin has the same membrane topology as MFN1 [Hu and Rapoport, 2016]), the authors also found that membrane tethering could occur upon continuous GTP hydrolysis.

The work by Qi et al. (2016) further implicates the GTPase domain as the site of mitochondrial tethering, rather than the C-terminal HR2 domain as proposed by Koshiba et al. (2004). Using these new insights, the authors suggest a new model of MFN-mediated mitochondrial fusion (Fig. 1 B). First, the helix bundle domains are extended, and upon GTP binding, the GTPase domains on opposing mitochondria dimerize. Upon GTP hydrolysis, conformational changes allow helix bundle 2 to rotate and bring helix bundle 1 and the GTPase domain into close proximity for interactions to occur. Finally, this brings the opposing membranes into close contact to drive outer mitochondrial membrane fusion. A similar model was earlier suggested by Escobar-Henriques and Anton (2013).

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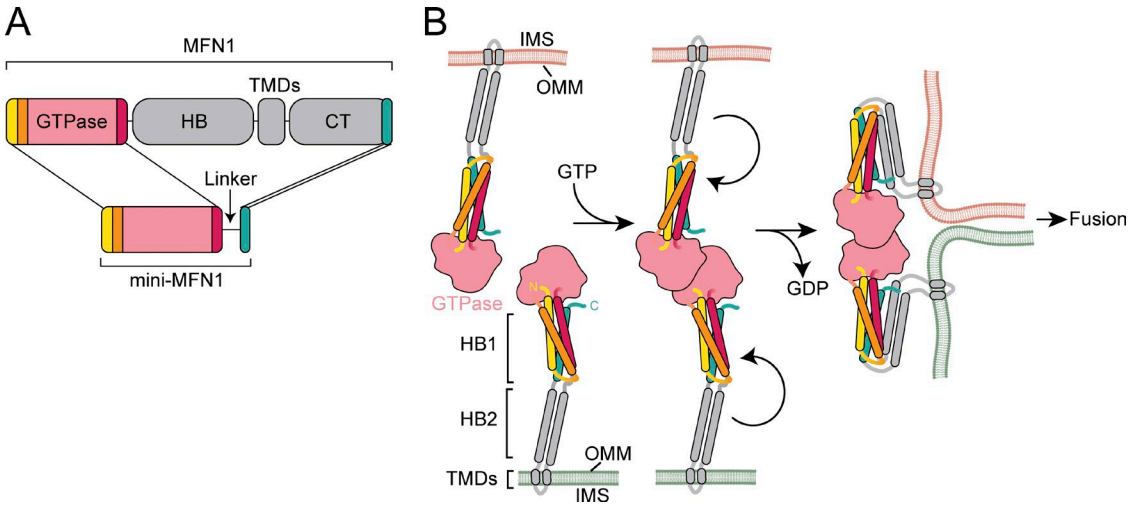

Figure 1. **Mini-MFN1 and a new proposed model of mitochondrial fusion mediated by MFN1.** (A) The domain structure of MFN1 and the synthetic construct mini-MFN1 used in the study by Qi et al. (2016). The N terminus of MFN1 consists of a GTPase domain followed by a helix bundle domain (HB), transmembrane domains (TMDs), and the cytosolic tail (CT). In the mini-MFN1 version, the GTPase domain and the end of the cytosolic tail are connected by a flexible linker. Colored regions represent the indicated structures shown in B. (B) A new model proposed to explain mitochondrial fusion. The GTPase domain is indicated in pink. The helix bundle 1 (HB1) is composed of two helices (yellow and orange) from the N-terminal extension of the GTPase domain plus an extended helix from the end of the GTPase domain (magenta) and a helix segment of the C-terminal tail (turquoise). Helices depicted in gray are proposed based on predicted structural and functional similarity with BDLP, whereas the position of the TMDs are based on topology studies. The outer mitochondrial membrane (OMM) and intermembrane space (IMS) are also indicated. The model suggests that upon GTP binding, the GTPase domains interact in trans to tether adjacent mitochondria. Upon GTP hydrolysis, conformational changes allow HB1 and HB2 to come together, bringing HB2 to the GTPase domain. In the final steps, the opposing membranes are brought into close proximity, resulting in fusion.

When pathogenic mutations in MFN2 that result in CMT2A were modeled onto the structure of mini-MFN1, many mutations either disrupted the GTPase activity of MFN1 directly or were at the interface of the GTPase and helix bundle 1. The GTPase activity of MFNs may be required for the interaction and tethering of GTPase domains in trans. Subsequent interactions between the GTPase domain and helix bundle 1 may bridge the gap between opposing mitochondrial membranes. Qi et al. (2016) suggest that this mechanism of membrane fusion could explain the pathogenicity in patients with dysfunctional MFN2.

Although this work has improved our understanding of the mechanism of MFN1-mediated mitochondrial fusion, many questions remain outstanding. First, what is the role of the helix bundle 2 in MFN action, and does this helix bundle 2 indeed behave like the trunk domain of BDLP? Second, given the sequence and structural similarities of MFN1 to the dynamins required for mitochondrial constriction and fission—DRP1 and DYN2 (Lee et al., 2016; Osellame et al., 2016)—how do MFNs promote the fusion, rather than division, of membranes? Further understanding of these questions will allow for a deeper appreciation of the mechanisms governing the complexity of membrane and organelle fusion.

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