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Mitochondria homeostasis: How do dimers of mitofusins mediate mitochondrial fusion?

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Summary

Mitochondria have high fusion and fission rates to maintain their size and number throughout the cell cycle. How is fusion mediated? New structural studies propose mechanisms by which the dynamin-like mitofusin proteins promote fusion of mitochondria.

Mitochondria are essential metabolic units of the cell, producing most of the ATP required for the cell's functions. In addition, mitochondria are required for numerous other processes — for example, the synthesis of essential cellular components, such as iron–sulphur clusters, or the regulation of induced cell death. As with powerhouses in countries, the size, number and location of mitochondria in cells are critical for their functions [1]. Mitochondria are highly dynamic organelles that constantly grow, split, fuse and move within a cell and the dynamics of mitochondria are therefore essential for regulating their number, size and location.

Among the essential processes that control mitochondrial dynamics, fusion and fission both involve proteins of the dynamin superfamily. Dynamin-like proteins (DLPs) have in common a highly conserved GTPase domain, one or two helical bundles that mediate assembly and/or nucleotide-induced conformational changes, and the ability to bind lipid membranes transiently or constitutively [2]. Dynamin-related protein 1 (DRP1), called DNM1 in yeast, contributes to mitochondrial fission: it binds to the outer membrane of mitochondria through adaptor proteins, such as MiD and FIS1, and, similar to dynamin, is then thought to constrict through an active twisting mechanism of its helical polymer [3,4]. The constriction of DRP1/DNM1 is not sufficient for fission, however; the ubiquitous dynamin 2 has been proposed to finalize fission after DRP1-mediated constriction [5].

Fusion of mitochondria also require DLPs, in particular optic atrophy 1 (OPA1; Mgm1 in *Saccharomyces cerevisiae*), which is located in the intermembrane space of mitochondria and mediates fusion of the inner membranes. Outer membranes are fused by the action of two other DLPs, mitofusins 1 and 2 (Mfn1 and Mfn2), which are located on the cytoplasmic face of the outer mitochondrial membrane. Mutations in OPA1 and mitofusins are often found in neurodegenerative diseases, such as dominant optic atrophy (DOA) and axonal Charcot-Marie-Tooth disease type A2 (CMTA2) [6]. In cells of DOA and CMTA2 patients, the disruption of mitochondrial fusion leads to a dramatic fragmentation of mitochondria, and in turn to defective metabolic activity. As a consequence, neurons are unable to renew themselves and therefore undergo degeneration.

Thus, OPA1 and mitofusins have been the subject of intense research to understand the link between mitochondrial dynamics and diseases. Two new manuscripts reporting the structure of Mfn1 [7,8] now shed light on the molecular mechanisms of mitochondrial fusion.

Structure of a Mfn1 Dimer

Mitofusins contain an amino-terminal GTPase domain, two helical bundles and two predicted transmembrane (TM) helices that are suggested to anchor the proteins in the outer mitochondrial membrane. In analogy with a related construct of dynamin [9], 'minimal' Mfn1 constructs with an internal deletion of the second helical bundle and the predicted TM region were generated [7,8]. These constructs are monomeric in the presence of GTP or GDP and in the absence of nucleotide, but they dimerize in the presence of GTPase transition state analogues.

Crystal structures of the minimal Mfn1 construct in the monomeric [7,8] and dimeric [7] state featured an extended dynamin-related GTPase domain of 250 amino acids and an adjacent four-helical bundle (Figure 1A). The latter is composed of helices α 1 and α 2 from the very amino-terminal region of Mfn1, helix α 3 from the region following the G-domain, and helix α 4 derived from the very carboxyl terminus which had been fused to α 3 via a linker.

Gao and colleagues [7] observed that dimerization of the GTPase domains is mediated via a conserved GTPase domain interface across the nucleotide-binding site. Dimerization via this so-called 'G-interface' is a conserved feature in all dynamin superfamily members [2] and often leads to activation of the GTPase activity. Accordingly, a protein concentration-dependent increase in the specific GTPase rates was observed for the Mfn1 construct, arguing for a similar mechanism of GTPase activation. Mutations in this G-interface led to impaired GTP hydrolysis and fragmented mitochondria, pointing to a crucial function of the G-interface in mitochondrial fusion. But to which step during membrane fusion does this dimer contribute?

Mechanistic Insights into Membrane Fusion

When analyzing the architecture of the Mfn1 dimer interface and the adjacent helical bundle, striking parallels with the bacterial dynamin-like protein (BDLP) became apparent (Figure 1B,C). In particular, the overall architecture of the GTPase domain and the first helical bundle (called the 'neck' in BDLP) is conserved in both proteins and differs from that of other DLPs. Furthermore, sequence comparisons indicate that the deleted stretch of sequence of the Mfn1 construct may form a second helical bundle corresponding to the trunk of BDLP, whereas the hydrophobic 'paddle' in BDLP may correspond to the predicted TM region of mitofusin. Based on this observation, BDLP might serve as a model for mitofusin-mediated membrane fusion.

In the GDP-bound and nucleotide-free form, BDLP was crystallized in a closed dimeric conformation, featuring a GTPase domain dimer that is highly similar to the newly described mitofusin structures (Figure 1B). Further dimerization contacts in BDLP are provided by the

trunk and the paddle (Figure 1B) [10]. In analogy with BDLP, Hu and colleagues [8] observed that deletion of the predicted TM sequences in Mfn1 abolished dimerization of the full-length protein, suggesting a similar role of the TM region in dimerization. Also, the orientation of the first helical bundle relative to the GTPase domain is almost identical in the closed BDLP structure and the Mfn1 construct.

A second BDLP conformation featuring a GTP- and membrane-bound oligomerized form has been described in a cryo-electron microscopy reconstruction [11]. A dramatic rearrangement of the helical bundles relative to the G-domain dimer was observed leading to an 'open' conformation, with the paddles inserting into the membrane (Figure 1C). Based on the striking structural similarities between BDLP and the Mfn1 construct, the authors of the current studies suggest that mitofusins undergo a similar, membrane-dependent opening mechanism.

What are the consequences of the new studies for understanding mitochondrial fusion? Low and Löwe [10] suggested that BDLP oligomerization on one membrane leaflet promotes high membrane curvature, as a prerequisite to undergo membrane fusion. This model is consistent with the structural conformations obtained for BDLP, but does not include interactions of BDLP molecules across two fusing membranes (Figure 2, right).

The authors of the current studies [7,8] envisage a different scenario for mitofusin, related to models of endoplasmic reticulum (ER) fusion mediated by the DLP atlastin [12,13]. Thus, GTPase domain dimers may initially form in trans across two adjacent membrane surfaces to allow tethering (Figure 2, left). A nucleotide hydrolysis-dependent power stroke may then pull the membranes together, allowing membrane fusion. This model is consistent with recent cryo-electron tomography analyses, in which elongated protein densities, possibly corresponding to open forms of the mitofusin molecules, were reported to contact each other across fusing mitochondrial membranes [14]. However, the resolution of this study was limited, precluding the elucidation of the exact protein geometries. It is also not clear to which step of such a fusion scenario the mitofusin dimer contributes (see alternatives in Figure 2, lower left).

In addition, an involvement of the carboxy-terminal mitofusin helix in mitochondrial tethering has been suggested [15]. In BDLP, this helix comprises an integral part of both four-helix bundles, with the hydrophobic residues of $\alpha 4$ contributing to the hydrophobic cores. Furthermore, no detachment of this helix has been observed so far. Therefore, this mechanism appears rather unlikely for BDLP, and probably also for mitofusins. On the other hand, it has been shown for atlastin that the carboxy-terminal helix directly inserts into the ER membrane and contributes to membrane remodeling [16]. Maybe, there are more, as yet unexplored, functions of carboxy-terminal helices in dynamin superfamily GTPases during membrane fusion.

Do Mitofusins Have a Prokaryotic Origin?

As described above, the structure of Mfn1 is very similar to that of BDLP, a prokaryotic protein. Because of the endosymbiotic origin of mitochondria, many mitochondrial proteins have prokaryotic ancestors, even though their genes are located in the cellular genome. The

most striking fact is that Mfn1 has a structure very similar to that of BDLP, while atlastins, guanylate-binding protein 1 (GBP1) and prokaryotic DLPs (Yjda from *Escherichia coli* and DynA_D1 from *Bacillus subtilis*) form a second subgroup within the dynamin family. In contrast, mitochondrial fission DLPs, such as DNM1/DRP1, belong to the third and largest subgroup of dynamins that also includes dynamins 1–3.

Thus, interestingly, mitochondrial fusion DLPs might have direct prokaryotic ancestors, whereas the fission DLPs appear to have eukaryotic ancestors. A missing piece of information is the structure of OPA1, a mitochondrial fusion DLP which seems quite divergent from dynamin, even though it forms membrane tubules that resemble the stereotypical dynamin-coated membrane tubules [17].

The future will tell whether fusion and fission DLPs have distinct structural features, allowing for functional predictions on the basis of their structure. Along with the beautiful published structures, a complete elucidation of the mechanism by which DLPs mediate membrane fusion may require additional functional *in vitro* assays, paired with electron microscopy assays that monitor distinct steps of the fusion reaction at high resolution.

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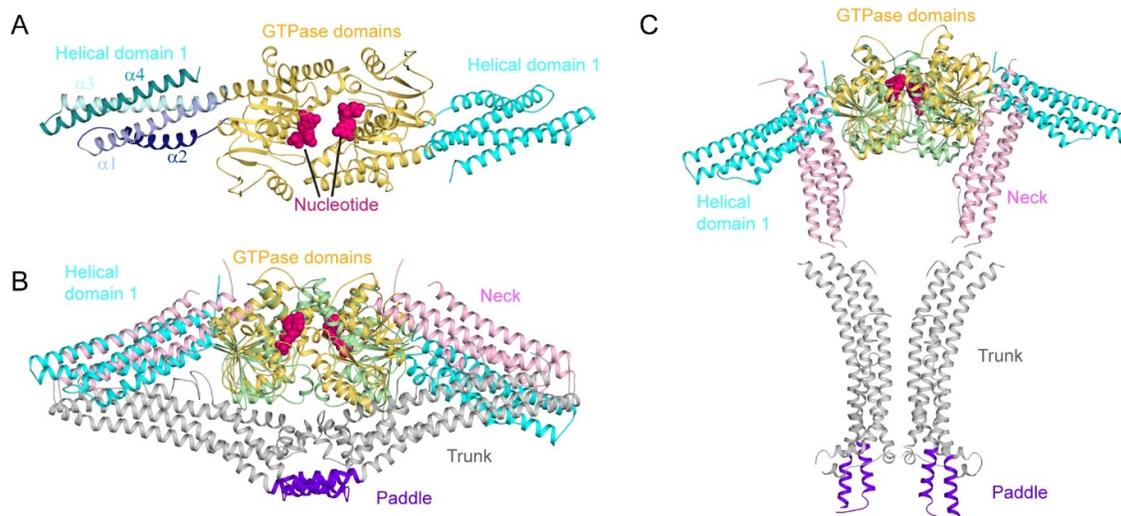


Figure 1: The structure of mitofusins.

(A) Top view of the mitofusin dimer (PDB 5GOM; [7]) crystallized in the presence of GDP-AIF4-: the GTPase domains dimerize via the canonical G-interface, whereas the helical domains have an extended conformation. (B) The left molecule of the mitofusin dimer was superimposed onto the closed GDP-bound dimer of BDLP (PDB 2J68; [10]): the left molecules almost perfectly superimpose, whereas the right molecules are slightly rotated with respect to each other. BDLP domains are specified on the right. (C) Superposition of the mitofusin dimer onto oligomerized GTP-bound BDLP (PDB 2W6D; [11]): whereas the GTPase domain dimer remains intact, the neck of BDLP undergoes a large-scale domain rotation towards the open form.

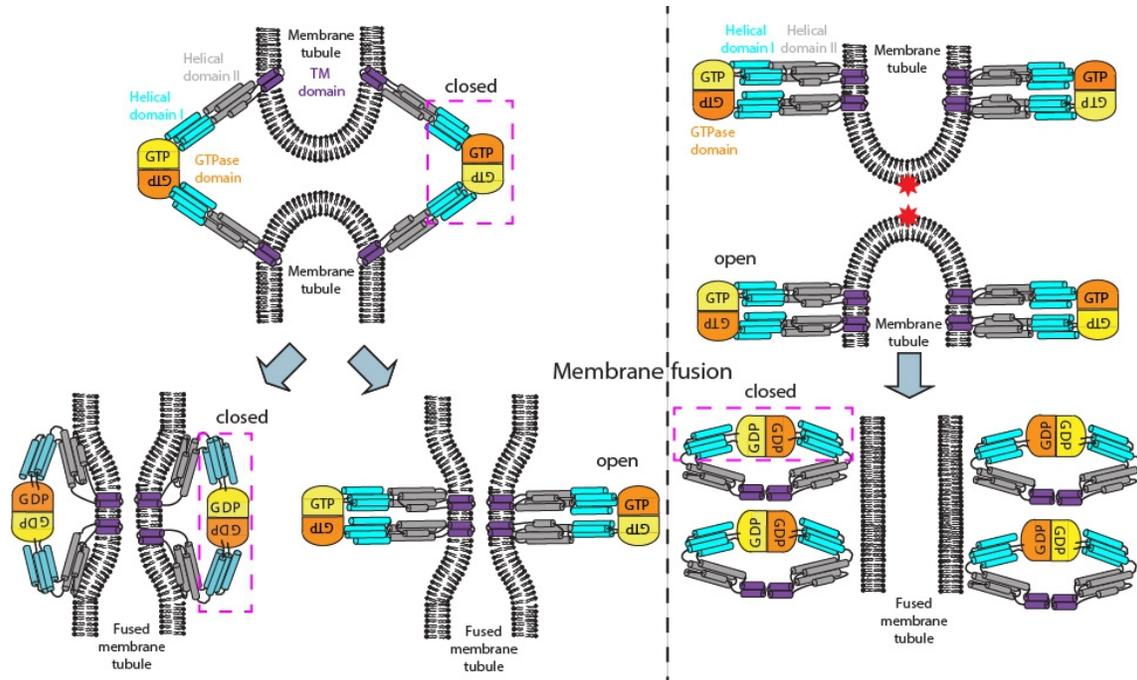


Figure 2: Putative structural fusion models of mitofusin. (Left) The GTPase domains of mitofusin molecules may contact each other across mitochondrial membrane tubules (for simplicity, only the outer mitochondrial membrane is depicted). A GTPase-dependent power stroke (lower left of left panel) or GTP-dependent oligomerization (lower right of left panel) may then lead to fusion of opposing membranes. (Right) According to the BDL model, mitofusins oligomerize and stabilize membranes of high curvature that are fusogenic (red star) and can therefore undergo membrane fusion. GTP hydrolysis may then lead to a 'closed' mitofusin conformation that is possibly detached from the membrane. Pink boxes indicate the GTPase domain dimer with the crystallographically observed closed conformation of the first helical bundle relative to the GTPase domain.