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From dynamin related proteins structures and oligomers to membrane fusion mediated by mitofusins

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ABSTRACT

Mitochondria assemble in a highly dynamic network where interconnected tubules evolve in length and size through regulated cycles of fission and fusion of mitochondrial membranes thereby adapting to cellular needs. Mitochondrial fusion and fission processes are mediated by specific sets of mechano-chemical large GTPases that belong to the Dynamin-Related Proteins (DRPs) super family. DRPs bind to cognate membranes and auto-oligomerize to drive lipid bilayers remodeling in a nucleotide dependent manner. Although structural characterization and mechanisms of DRPs that mediate membrane fission are well established, the capacity of DRPs to mediate membrane fusion is only emerging. In this review, we discuss the distinct structures and mechanisms of DRPs that trigger the anchoring and fusion of biological membranes with a specific focus on mitofusins that are dedicated to the fusion of mitochondrial outer membranes. In particular, we will highlight oligomeric assemblies of distinct DRPs and confront their mode of action against existing models of mitofusins assemblies with emphasis on recent biochemical, structural and computational reports. As we will see, the literature brings valuable insights into the presumed macro-assemblies mitofusins may form during anchoring and fusion of mitochondrial outer membranes.

1. Introduction

Mitochondria are double membrane organelles that govern cellular energy generation and are essential to mediate apoptosis and cell signaling. During a cell life span, mitochondria assemble into a dynamic network that interconvert between elongated tubules and small fragmented units to maintain cell viability and mitochondrial function. The morphology and dynamics of this network are shaped and triggered by movement of mitochondria along cytoskeletal tracks and are associated with tightly regulated cycles of fission and fusion of mitochondrial membranes [1–5]. Mitochondria fragment to isolate damaged or aging sections of the network [6,7], move to cell locations which require high ATP production [8–10] or to ensure mitochondrial genome propagation from mother to daughter cells [11–14]. Conversely, mitochondrial tubules fuse together to homogenize the proteomic content of healthy mitochondria and face the gradual accumulation of mutations in mitochondrial DNA [15–17]. Mitochondrial fusion also maximizes energy production and can protect cells from autophagy during starvation [18,19]. Deciphering the mechanistic cues of mitochondrial fission and fusion is therefore of primary physiological importance.

The machineries that control mitochondrial dynamics belong to the membrane-bound large GTPases of the Dynamin-Related-Proteins (DRPs) superfamily which are implicated in membrane remodeling processes. Mitochondrial fission DRPs include Mammalian DRP1 and its yeast orthologue Dnm1 [20–22]. Mitochondrial fusion encompasses two sets of DRPs that cooperatively mediate the fusion of outer and inner mitochondrial membranes. Fusion of outer mitochondrial membranes (OMMs) involve the mitofusin subclass of DRPs that include MFN1 and MFN2 in mammals and Fzo1 in yeast [23–26] while fusion of inner mitochondrial membranes (IMMs) is mediated by Mgm1 in yeast and OPA1 in mammals [27–29]. Notably, alterations in OPA1 are causal for autosomal optic atrophy and mutations in human MFN2 are associated with the onset of neurodegenerative diseases including Charcot-Marie-Tooth type 2a disease (CMT2A), which is characterized by degeneration of long peripheral nerves [30–32].

The structure and function of DRPs are tailored to meet the successive energy-demanding intermediate stages of membrane remodeling processes such as tubulation, fission and fusion [33,34]. For this purpose, DRPs harbor two intrinsic features: the ability to attach to membranes and the capacity to auto-oligomerize into macro-assemblies. In

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agreement with these features, fission DRPs bound to GTP such as DRP1/Dnm1 are well established to assemble into macro-molecular spirals that wrap around the membrane compartments to which they are recruited [35–39]. The power stroke from GTP hydrolysis within the spiral induces its conformational switch resulting in reduced diameter, membrane constriction and ultimate fission [40–42]. On the other hand, most fusion DRPs including mitofusins, oligomerize in *cis* (on the same membrane) and in *trans* (on opposite membranes) to tether opposite membranes. GTP hydrolysis within fusion DRPs also induce conformational changes that bring membranes closer together in a ‘docked’ stage prior to their fusion. Yet and in contrast to fission DRPs, macro-assemblies of fusion DRPs remain elusive. In this review, we will thus present an overview of several DRPs before highlighting distinct oligomerization assemblies of selected fission and fusion DRPs. We will then focus on mitofusins to discuss recent biochemical, structural and computational data bringing insights into the presumed macro-assemblies they can form during anchoring and fusion of mitochondrial outer membranes.

2. Dynamin related proteins: function and architecture

The founder of the dynamin superfamily is the mammalian dynamin (Dyn1) which is involved in the separation of clathrin-coated vesicles from the plasma membrane during endocytosis [43,44]. Additional factors identified based on sequence and structural homology with Dyn1 was further classified into two subfamilies including classical dynamins and DRPs, also known as dynamin like proteins (DLPs). The first identified DRP was the Myxovirus resistance protein MxA [45] and its yeast homolog Vps [46,47] involved in virus resistance and fission of endosomal membranes, respectively. The functional repertoire of DRPs in eukaryotes then diversified to encompass distinct cellular processes

(Fig. 1). Atlastin (Atl) (Sey1 in yeast) mediate fusion of ER membranes [48,49] while Drp1/Dnm1 mediate fission of mitochondria [20–22] but also of peroxisomes [41]. As also mentioned above, the mitofusins MFN/Fzo1 on one hand [23–26] and OPA1/Mgm1 [27–29] on the other hand, promote respective fusion of mitochondrial outer and inner membranes [50,51]. DRPs also include the antimicrobial Guanylate-Binding Proteins (GBPs) which serve for viral surveillance and sequestration [52]. Importantly, alterations of several mammalian DRPs are causative in numerous pathologies and severe neurodegenerative disorders such as Parkinson's disease, Charcot Marie Tooth (CMT) type 2A, autosomal optic atrophy or spastic paraplegia (HSP/Strümpell–Lorrain syndrome) [31,53–55].

Notably, DRPs are also widespread in plants. One highly interesting property of plant cells is that they harbor both mitochondria for oxidative phosphorylation and chloroplasts for photosynthesis [56]. The two organelles are subject to similar membrane remodeling processes but thylakoids within chloroplasts also undergo fission and fusion of their photosynthetic membranes. Plant DRPs are currently best characterized in *Arabidopsis thaliana* where ARC5 also known as DRP5B triggers chloroplast fission through a mechanism reminiscent of mitochondrial fission (Fig. 1) [57]. Intriguingly, the sole mitofusin-like factor encoded by plant genomes, Fzl, regulates fusion of thylakoid membranes in plant and algal cells [58–60]. Nonetheless, Fzl does not influence mitochondrial dynamics and the factors that trigger mitochondrial fusion in plant cells remain to be discovered.

Fzl is the closest homolog of mitofusins from fungi and metazoans but also for the Bacterial Dynamin-Like Protein (BDLP) in the cyanobacteria *Nostoc punctiforme*. Since the pioneering elucidation of BDLP structure and in vitro analysis [61,62], structural reports on bacterial DLPs are evolving and include DynA, DLP1 and DLP2 [63–65]. Unlike eukaryotic and plant DRPs, most of their functions remain however to be

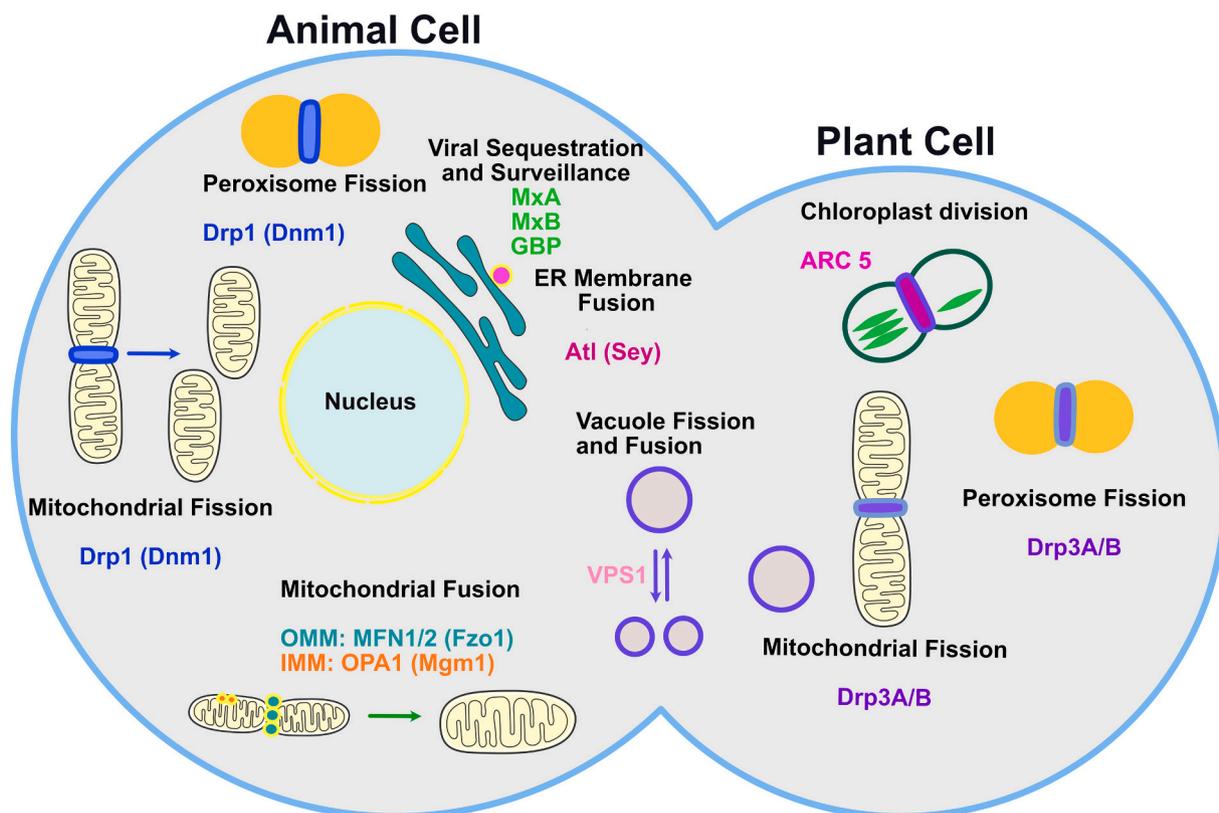


Fig. 1. Schematic illustration of selected DRPs function in animal and plant cells detailed in the text. Drp1 is involved in mitochondrial and peroxisome scission [20–22,41]. ARC5 is involved in chloroplast division [57]. Atl/Sey1 are involved in ER fusion [48,49], whereas MFNs/Fzo1 fuse the outer [23–26] and OPA1/Mgm1 [27–29] the inner mitochondrial membranes. VPS1 [46,47] is involved in vacuole fission and fusion. MxA [45] and GBP [52] are involved in viral sequestration and surveillance.

precisely defined but are suspected to include cell division during sporulation, fusion of photosynthetic membranes, bacterial outer membrane vesiculation or molecular sutures for the repair of lipid bilayers [63–65].

DRPs and ‘classical’ dynamins gather the same kind of large GTPase domain (~300 aa) in addition to two or more characteristic regions. DRPs are nonetheless distinguished by the absence of the Proline Rich Domain (PRD) found in classical dynamins [30] (Fig. 2). Some DRPs are integral membrane proteins thanks to transmembrane regions that promote insertion into cognate lipid bilayers. Other DRPs are peripheral membrane proteins that transiently attach to the membrane using lipid-interacting modules such as the B insert or paddle regions (Fig. 2). Regardless of how they attach to the membranes, the GTPase activity of DRPs is usually stimulated by their auto-oligomerization [66]. In fact, the highest distinguishing features of DRPs that allow their oligomerization and stimulation of their GTPase activity are helical regions composed of a Middle domain and a GTPase-effector domain (GED). The Middle domain is also known as the 3HB (Helix-Bundle), the 4HB or the BSE (Bundle Signaling Element) whereas the GED is also called the Stalk or the Trunk (Fig. 2). DRPs auto-interact both through their GTPase domain and through their GED which represents an important regulatory interaction site [30]. This drastically contrasts with small GTPases that require specific GTPase Activating Proteins (GAPs) and Guanine nucleotide Exchange Factors (GEFs) [67,68]. Consistent with this mechanistic distinction, DRPs have a micromolar affinity for nucleotides which is lower than the nanomolar affinity of small GTPases [69]. DRPs also have a higher basal GTP hydrolysis rate which is further stimulated upon auto-assembly through their GTPase/Middle domain and their GED [63,70,71]. Notably, DRPs often work in synergy with co-factors that favor their oligomerization capacity and/their recruitment to membranes. GTP hydrolysis also drives canonical conformational changes in their GTPase domain and their helical regions, leading to the remodeling of lipid bilayers.

3. Overview of selected DRP oligomers

Although auto-oligomerization is a common feature among DRPs,

the oligomerization properties of each DRP can strongly vary in terms of stoichiometry or binding interface. DRPs often assemble into homo-oligomers, but can also associate into hetero-oligomers between two distinct DRPs as we will see below with DLP1 and DLP2 [63]. Homo and hetero oligomers can be further classified according to the number of interacting units where the stoichiometry can involve dimers, trimers or tetramers that further assemble into tubules or rings. For instance, dimeric assemblies include Atl [72] or mitofusins [73,74], whereas ring like and helical filament structures are adopted by MxA [75], Drp1 [37,76], EHD2 [77], BDLP [62] or Mgm1/OPA1 [78,79]. DRP interacting units can also vary based on the two main interfaces employed for assembly, the nucleotide-dependent assemblies through the GTPase domain and the nucleotide-independent assemblies predominantly mediated through the GTP effector domain. Last but not least, oligomeric species vary based on the position of DRPs relative to membranes. Oligomers formed between DRPs belonging to the same membrane are referred as *cis* oligomers whereas those formed between DRPs belonging to two opposite membranes are referred as *trans* oligomers.

DRPs that mediate membrane fission such as DRP1 and Dnm1 represent the most accurate model to illustrate the ability of DRPs to oligomerize into macromolecular structures. DRP1 and Dnm1 include the GTPase, BSE and Stalk domains as well as a flexible B-insert, which favors anchoring to membranes (Fig. 2). Both DRPs engage in Stalk-Stalk interactions in the absence of nucleotides to generate ‘back-to-back’ oligomers (Fig. 3A) [35,36,76,80]. GTP binding was also found to trigger G-G interactions to form ‘head-to-head’ associations [76,81–83]. Electron microscopy studies demonstrated that Stalk-Stalk interactions allows their assembly into large helical oligomers that warp around membranes (Fig. 3B) [35,39,76,80]. Within these helical assemblies, G domains of adjacent turns transiently dimerize to mediate a GTPase-dependent power stroke which pulls adjacent filaments along each other leading to membrane constriction (Fig. 3B). It was further demonstrated that DRP1 could form closed rings, but only through the formation of a linear oligomer that depends on the DRP1 receptor MID49 [80].

Atlastin (Atl) is the best example of a DRP *trans* dimer that could take place between two opposite ER membranes. In terms of domain

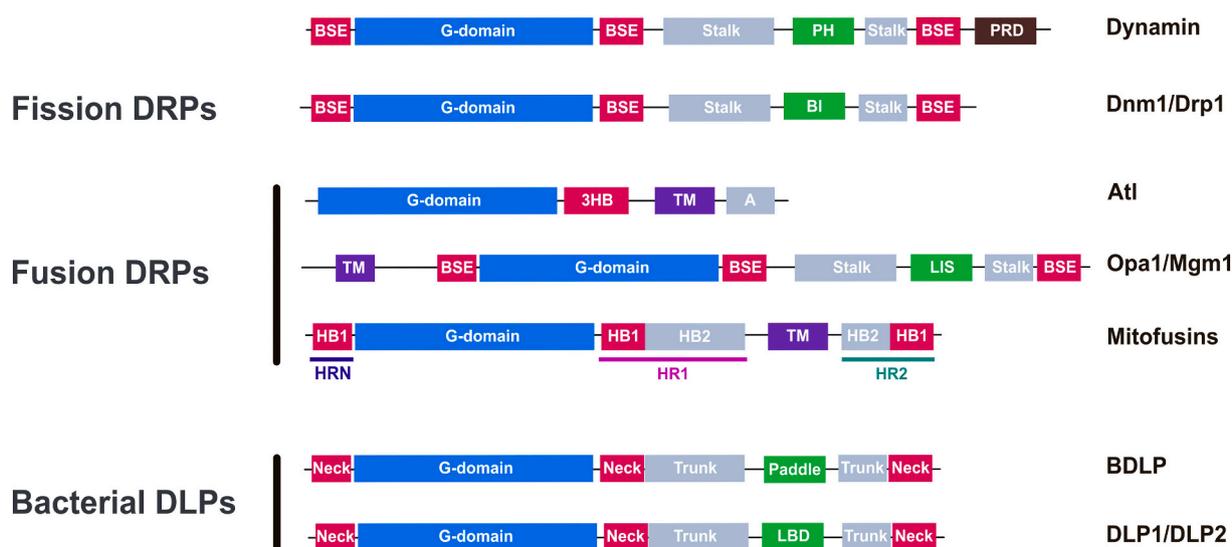


Fig. 2. Schematic representation of the domain organization of Dynamins and selected DRPs. Domains that share structural/functional relations are depicted in the same color. Abbreviations: G-domain: GTPase domain; BSE: Bundle Signaling Element; PH: Pleckstrin Homology domain; PRD: Proline Rich Domain; BI: B insert; LBD: Lipid Binding Domain; LIS: Lipid Interacting Stalk; TM: Transmembrane domain, A: Amphipathic helix; HRN: N-terminal Heptad Repeat domain present in the yeast mitofusin Fzo1 but not in the mammalian mitofusins MFN1 and MFN2; HR1: Heptad Repeat domain 1; HR2: Heptad Repeat domain 2; HB1: Helix Bundle 1; HB2: Helix Bundle 2. Note that HB1 in mitofusins is identified based on the crystal structure of truncated MFNs and contains helices from the N-terminal part, the HR1 domain and the extreme C-terminal half the HR2 domain, while HB2 of mitofusins is speculated based on the BDLP structure and is expected to include the complementary helices of the HR1 and HR2 domains. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

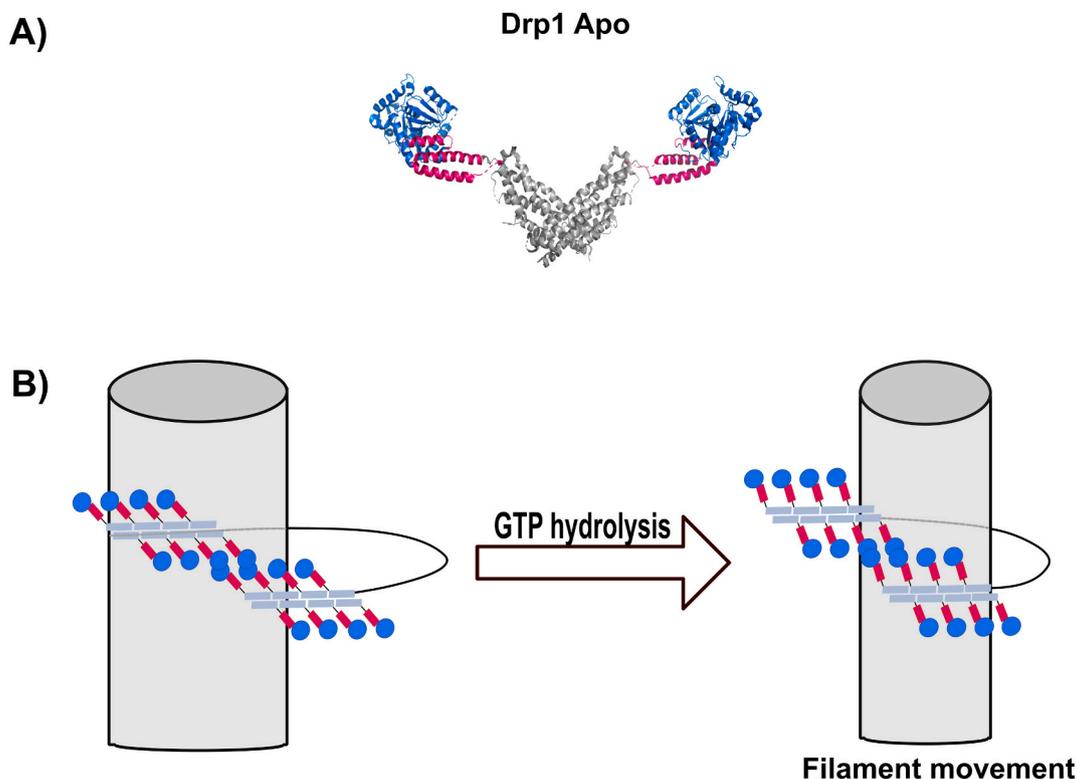


Fig. 3. (A) Crystal structure of Drp1 dimer in the absence of nucleotide (PDB: 4BEJ) [35]. (B) Schematic representation of Drp1 membrane constriction model [35]. In the absence of nucleotides, Drp1 assembles into a double layer helical oligomers built by back-to-back dimers that stack on each other via stalk-stalk interactions leaving the GTPase domain located on one side of the filament. Only one filament is shown on each side and the B insert is omitted for clarity. The G domains from two filaments can dimerize at helical turns. The power stroke from GTP hydrolysis is expected to induce conformational changes within the filament and eventually membrane constriction.

organization, Atl is composed of a cytosolic N-terminal G-domain connected to a 3-Helix Bundle domain (3HB), followed by two closely spaced transmembrane (TM) segments and a cytosolic C-terminal tail (Fig. 2). Upon nucleotide binding, the GTPase-domain of Atl was found to form two distinct G-homodimers that differ with orientation of the 3HB domain (Fig. 4A) with no evidence of macro-assembly formation [72,84–86]. Supported lipid bilayer experiments with Atl also

demonstrated that the DRP can engage in *cis* G-dimers upon addition of nucleotide analogs [72]. This led to the hypothesis that GTP hydrolysis may be essential to dissociate pre-existing *cis* G-dimers and generate a pool of monomers competent to engage in *trans* G-dimerization to promote the attachment of ER membranes (Fig. 4B) [72]. GTP hydrolysis within the *trans* dimer subsequently induces a swing in the 3HB domains dragging the opposite membranes in close apposition to allow their

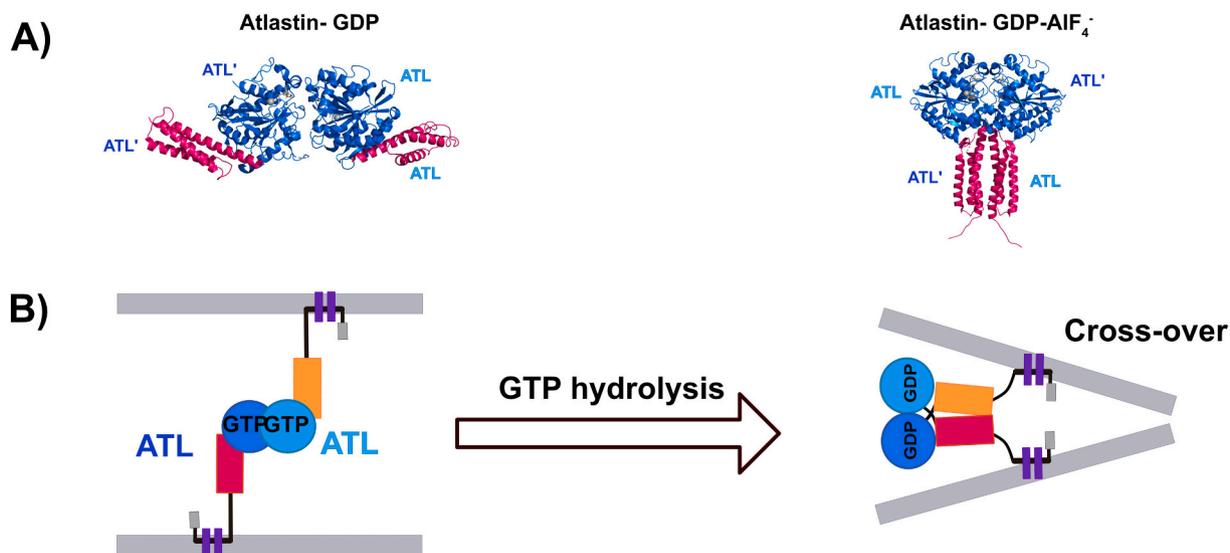


Fig. 4. (A) crystal structures of ATL G-G-dimers in open conformation in complex with GDP (PDB: 3QOF) [84] and closed conformation in complex with GDP-AIF₄⁻ (PDB: 4IDQ) [85]. (B) Fusion model for Atl. Membrane attachment is mediated via *trans* G-dimers across ER membranes, which upon GTP hydrolysis, transition toward a cross-over conformation bringing opposing membranes into close proximity. Domains are colored as described in Fig. 2.

ultimate fusion [72,84–86].

This Atl model of DRP oligomerization appeared somewhat simple when the burst of structural data on OPA1 and Mgm1 was published [87–90]. The DRPs involved in fusion of mitochondrial inner membranes include an N-terminal transmembrane (TM) domain proximal to the GTPase domain followed by the two central helical regions BSE and Stalk as well as a lipid-interacting module at the end of the molecule (Fig. 2). During their import into mitochondria, Mgm1 and OPA1 undergo a partial proteolytic processing yielding a TM-containing long form (L-Mgm1/L-OPA1) and a soluble short form (s-Mgm1/s-OPA1) which are both essential for fusion [91–96]. Truncated short forms only including the GTPase and BSE domains (OPA1-MGD and Mgm1-MGD) assemble into ‘head-to-head’ G-dimers in the presence of transition state analogs (Fig. 5A) [89,90]. Truncated s-Mgm1 isoform from *Chaetomium thermophilum* and s-OPA1 were also generated and found to assemble into nucleotide independent dimers mediated through Stalk-Stalk (‘back-to-back’) interactions (Fig. 5B-i) which further assembles into tetramers mediated by Stalk-Stalk and BSE-Stalk interactions (Fig. 5B-ii and iii) [87]. These ‘back-to-back’ oligomers are reminiscent of those formed through BSE-Stalk interactions (see above) with fission DRPs [78,79]. Consistent with this, purified s-Mgm1 and s-OPA1 have the ability to tubulate membranes by forming helical filaments consisting of back-to-back building blocks that wrap around the membrane through head-to-head interactions promoted by GTP binding [87,88]. These filaments may assemble on opposing membrane buds to stabilize the membrane curvature at the fusion site (Fig. 5B-iv). Nucleotide-state transition was hypothesized to induce membrane constriction/expansion through conformational changes within the oligomer. Notably, these oligomers were also formed on the luminal side of liposomes (Fig. 5B-iii) [87] which led to the suggestion that OPA1 and Mgm1 may employ this mechanism to promote formation of the mitochondrial cristae as was previously demonstrated [51,97] (Fig. 5B-v). Recent structural data on truncated s-Mgm1 from *Saccharomyces cerevisiae* in complex with GDP revealed a third trimeric ‘head-to-tail’ oligomer generated through interactions between the G-domain and the ‘Stalk/LIS’ region (Fig. 5C-i). This trimer can further assemble in an anti-parallel fashion via its Stalk and BSE into a hexamer generating a concave G-LIS domain interface [89]. Notably, GTP binding inhibits formation of this trimer [89]. It was therefore proposed that G-G interaction does not directly contribute to the fusogenic complex. Instead, the trimer assembles with its concave surface on the IMM into spirals promoting membrane curvature and inducing spontaneous fusion tips (Fig. 5C-ii) [89]. Nonetheless, this Mgm1-mediated fusion model remains to be validated. Regardless of these conflicting models, these reports clearly unravel the formation of fusion macro-assemblies that go beyond simple *trans* G-dimers.

Reports on bacterial DRPs have represented yet another keystone in our understanding of DRPs oligomerization properties [61,62]. The Bacterial Dynamin Like Protein (BDLP) from the cyanobacteria *Nostoc punctiforme* is closely related to the plant Fzl and to the yeast mitofusin Fzo1. While its function remains to be precisely defined, BDLP is assumed to promote membrane repair, presumably through fusion [61–63,65]. BDLP includes an HB1/BSE/Neck region, an HB2/Stalk/Trunk domain and an helical paddle that associates with lipids (Fig. 2). Crystal structures of BDLP in the apo or GDP-bound form revealed a compact structure where the GTPase domain and the Neck (equivalent to the BSE or HB1 in OPA1/Mgm1 or mitofusins, respectively) fold onto the Trunk (equivalent to the Stalk or HB2 in OPA1/Mgm1 or mitofusins, respectively) and paddle (Fig. 6A). This closed conformation is soluble and has the ability to form G-dimers [61]. BDLP bound to the non-hydrolysable analog GMP-PNP binds to membranes. Cryo-EM analysis unraveled an open conformation where the GTPase and the Neck are separated from the Trunk and the paddle that inserts into lipid bilayers (Fig. 6A) [62]. This ‘opened’ BDLP homodimerizes both through the G-domain and the Trunk but also has the ability to form a macro-molecular lattice that tubulates liposomes (Fig. 6B) [62]. Given its homology to

Fzo1, BDLP has been an outstanding resource to predict conformational switches and oligomerization properties of mitofusins.

The complexity of DRPs oligomerization properties was further depicted with a nucleotide-independent hetero-tetramer formed between two DRP isoform pairs from *Campylobacter jejuni* [63]. Cj-DLP1 and Cj-DLP2 are composed of the same motives found in BDLP with a modified lipid binding domain (Fig. 2) located at the tip of the fully folded proteins (Fig. 7) [63]. Both DRPs assemble into a tetramer composed of a central DLP2 dimer formed through Trunks and GTPase domains interactions, which is flanked on each side by a DLP1 unit associated to the Neck of DLP2 through the N-terminal region (Fig. 7A) [63]. Equimolar amounts of Cj-DLP1 and Cj-DLP2 incubated with liposomes in the absence of nucleotides, induced strong membrane tethering suggesting that the tetramer may promote membrane anchoring (Fig. 7B) [63]. Whether nucleotides may promote higher order assemblies of both DRPs which would ultimately trigger membrane fusion will be highly relevant to assess in the future.

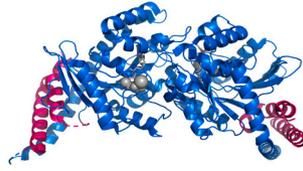
4. Properties and architecture of mitofusins

As compared to all models above, the precise oligomerization properties of mitofusins remain overall to be discovered. This is mainly due to the unresolved challenge of producing and purifying full-length and functional proteins. Nonetheless, the function and properties of mitofusins have been deeply studied since their discovery in 1997 [98] which provides many insights on how these factors could associate to promote anchoring and fusion of mitochondrial outer membranes. All mitofusins are characterized by an N-terminal GTPase domain and two C-terminal HR1 and HR2 heptad repeat domains that sandwich a transmembrane region (Fig. 8A) [24,25,99–102]. The yeast mitofusin Fzo1 includes an additional HRN domain upstream the GTPase while MFN2 harbors a proline rich domain downstream the HR1 domain that may be required for protein-protein interactions [101,103]. Importantly, integrity of all these domains is essential for mitochondrial fusion [74,100,104–106].

The main distinction between mammalian and yeast systems lies in the requirement for two distinct mitofusins in humans whereas a single one is sufficient to mediate mitochondrial fusion in fungi. In cells lacking either MFN1 or MFN2, either mitofusin can functionally replace the other, although with different efficiency, indicating that both are required for optimal mitochondrial fusion [107]. Consistent with this, depletion of *MFN1* lead to highly fragmented mitochondria, while depletion of *MFN2* generates big mitochondrial fragments that cluster perinuclearly, indicating that both mitofusins have distinct and specific functions in mitochondrial fusion [23,104,107,108]. Further confirming their functional distinction, MFN1 and MFN2 have significantly distinct tethering and GTPase hydrolysis activities [108]. For instance, both MFN1 and MFN2 can dimerize through their GTPase domain but MFN2 has a much tighter G interface than MFN1 and remains dimerized even after GTP hydrolysis [73,104]. Moreover, while MFN1 and MFN2 interact together [23] and with MIEFs (MIEF1/2) which act as hubs for regulating the balance between fusion and fission in mammalian cells [109], both mitofusins have distinct and unique interaction partners. Despite OPA1 interacts with both mitofusins [110], MFN1 but not MFN2 is required for mitochondrial fusion completion driven by OPA1 [28]. MFN2 interacts with the Bcl-2 family members Bak and Bax while MFN1 does not [111,112]. Accordingly, mutations in MFN2 are causal in the CMT2A disease [31] but mutations in MFN1 causing CMT2A are still to be identified. Interestingly, domain swap data on MFN1 and MFN2 demonstrated that introducing the N-terminal region of the MFN1 HR1 into MFN2 restores mitochondrial fusion in cells lacking MFN1 [113]. Moreover, MFN1 was found to assemble into 450 kDa oligomers in the presence of transition state analogs of GTP, while MFN2 does not seem to have the same capacity to form such high molecular weight oligomers [113]. MFN1 and MFN2 share ~80 % sequence similarity [107], suggesting that their functional differences lie in their 20 % dissimilarity.

A)

Sc Mgm1- MGD
Head-to-Head



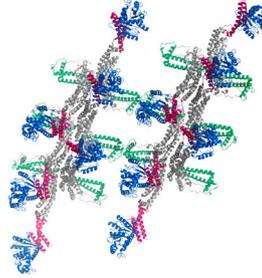
B)

i) Ct s-Mgm1
Back-to-Back



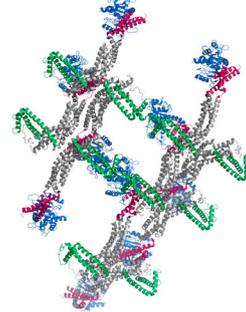
Ct s-Mgm1 assembly for outer surface

ii)

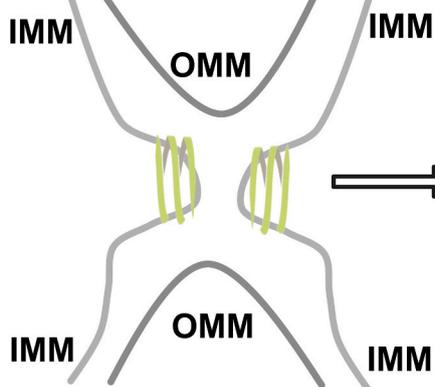


Ct s-Mgm1 assembly for inner surface

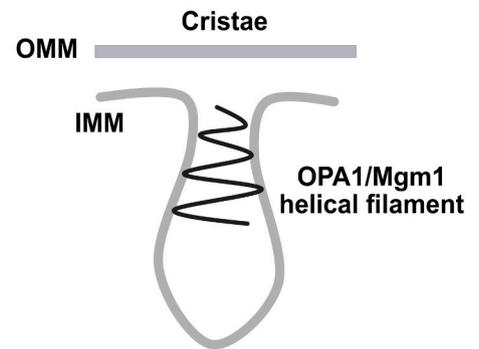
iii)



iv)

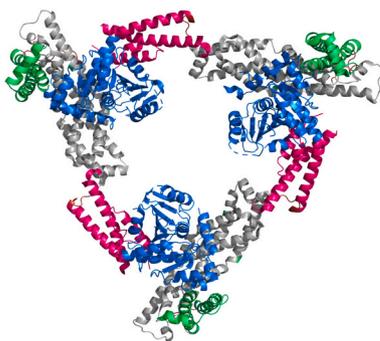


v)



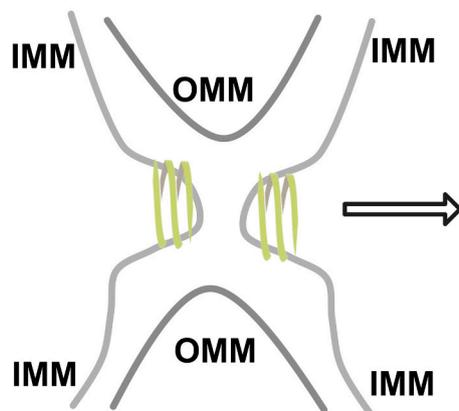
C) i)

Sc s-Mgm1- GDP
Head-to-Tail



ii)

Concave assemblies



(caption on next page)

Fig. 5. (A) Structure of Mgm1-MGD from *S. cerevisiae* forming G-G dimer, termed 'head-to-head' assembly, in complex with GDP (PDB: 2X2F) [89]. (B) Structures of truncated s-Mgm1 from *C. thermophilum* [87]. (i) Crystal structure of s-Mgm1 dimer that assembles via a central Stalk-Stalk interaction termed 'back-to-back' in the absence of nucleotide (PDB: 6QL4); Cryo-EM structures of s-Mgm1 helical assemblies decorating the (ii) outer (PDB: 6RZU) and (iii) inner (PDB: 6RZW) surface of lipid tubules. (iv) Tetramer-based IMM membrane fusion model. During inner-membrane fusion, Mgm1 or OPA1 helical filaments may assemble on opposing membrane buds to stabilize the membrane curvature and fusion pore formation. Upon membrane tethering, deposits of s-Mgm1 move toward each other, resulting in lipid mixing that can expand to complete IMM fusion. (v) Model for Mgm1/OPA1 mediated cristae formation. Mgm1 filaments may assemble into left-handed helical filaments to constrict the cristae junction in a GTPase-dependent fashion. Alternatively, they may assemble into right-handed helical filaments that expand the cristae volume to prevent their collapse. (C) s-Mgm1 from *S. cerevisiae* [89]. (i) Structure of s-Mgm1 trimer that is built by G interactions with stalk/LIS termed 'head-to-tail' in the presence of GDP (PDB: 6JSJ). (ii) Trimer-based IMM membrane fusion model. During inner membrane fusion, 'head-to-tail' assemblies bind to the IMM via their concave G and LIS interface. They further assemble into spirals which stack in an anti-parallel fashion mediated by BSE and Stalk interactions promoting membrane curvature and inducing spontaneous fusion tips. When tips from opposing membranes encounter each other, lipid mixing results in a fusion pore that can expand to complete IMM. Domains are colored as described in Fig. 2.

In terms of topology, the sole yeast mitofusin Fzo1 adopts a U-shaped bipartite transmembrane (TM) region that spans the outer membrane twice exposing its N- and C-terminal extremities in the cytoplasm (N_{out} - C_{out}) (Fig. 8B) [114]. Originally, MFN1 and MFN2 were thought to adopt the same topology as that of Fzo1 [99,115] but recent comparative analyses and phylogenetic reconstructions have revealed that human mitofusins may rather include a single transmembrane helix [116]. It has thus been proposed that the C-terminal HR2 domain of MFNs resides in the mitochondrial intermembrane space rather than in the cytosol (N_{out} - C_{in}) (Fig. 8B) [116]. Yet, C-terminal tagging forces the N_{out} - C_{out} topology of MNFs but totally restores mitochondrial fusion in the absence of MFN1 or MFN2 [116]. Structures of truncated MFN1 and MFN2 indicate that a significant portion of the HR2 contributes to the formation of the 4HB HB1/Neck of mitofusins rather supporting an N_{out} - C_{out} topology [73,74,104]. It is thus likely that MFN1 and MFN2 have the ability to adopt two distinct topologies. Further work will be required to investigate their distinct functions but one could speculate that these topologies could act as co-factors for each other.

In this regard, Ugo1 is a 3 transmembrane domains co-factor of Fzo1 localized on mitochondrial outer-membrane [117,118]. Ugo1 interacts both with Fzo1 and with Mgm1 and is essential for homotypic fusion of both outer and inner membranes suggesting that this carrier-like protein may coordinate sequential fusion of mitochondrial membranes [119,120]. Furthermore, Fzo1 has been proposed to form *cis*-

homodimers in a Ugo1 and GTP dependent manner which may prime initiation of mitochondrial tethering [121]. SLC25A46 is a mammalian ortholog of Ugo1 that interacts with both MFN2 and OPA1 [122,123]. However, knockdown of SLC25A46 increases mitochondrial fusion indicating that the role of Ugo1 is not conserved from yeast to metazoans [123].

Besides the mitochondria, mitofusins have unique subcellular expression patterns. For instance, MFN2 (but not MFN1) is localized in the Endoplasmic Reticulum (ER)/Sarcoplasmic Reticulum (SR) and regulates tethering of ER/SR to the mitochondria without driving heterotypic fusion [124–127]. Similarly, Fzo1 was recently found to co-localize with peroxisomes and to promote peroxisome–mitochondria contacts [128]. To mediate these contacts, MFN2 or Fzo1 presumably engage in *trans* oligomerization, however this is beyond the scope of this review.

5. Mitofusin oligomers

Mitofusins oligomerize in *cis* and tether opposing lipid bilayers through oligomerization in *trans* [23,107,108,116,121]. In mammals, redox-mediated disulfide modifications may drive the assembly of homo and hetero-oligomeric mitofusin species in *cis* [116]. In yeast, Fzo1 homodimers may assemble in a Ugo1 and GTP dependent manner [121]. How mitofusins oligomers in *cis* and *trans* coordinate to trigger fusion of

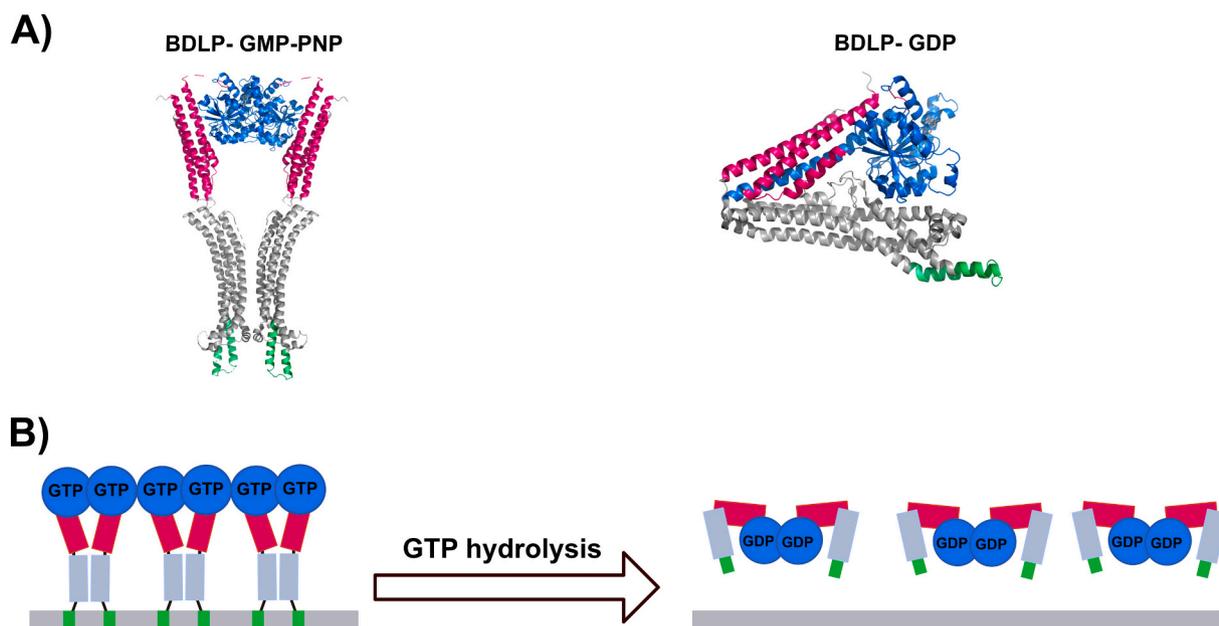


Fig. 6. (A) Cryo-EM structure of the open conformation BDLP dimer in complex with GMP-PNP (PDB: 2W6D) and crystal structure of closed conformation BDLP in complex with GDP (PDB: 2J68) [61,62]. (B) Oligomerization model of BDLP. In the GTP bound state, BDLP inserts into the membrane probably via the paddle region and polymerizes on the surface forming G-G dimers that further self-associate via their G-effector domain in a parallel fashion generating high curvature. GTP hydrolysis converts BDLP into a compact conformation which is released from the membrane. If the two membranes belong to the same surface, this results in fission. If they belong to two different surfaces (two vesicles, for example), the process results in fusion. Domains are colored as described in Fig. 2.

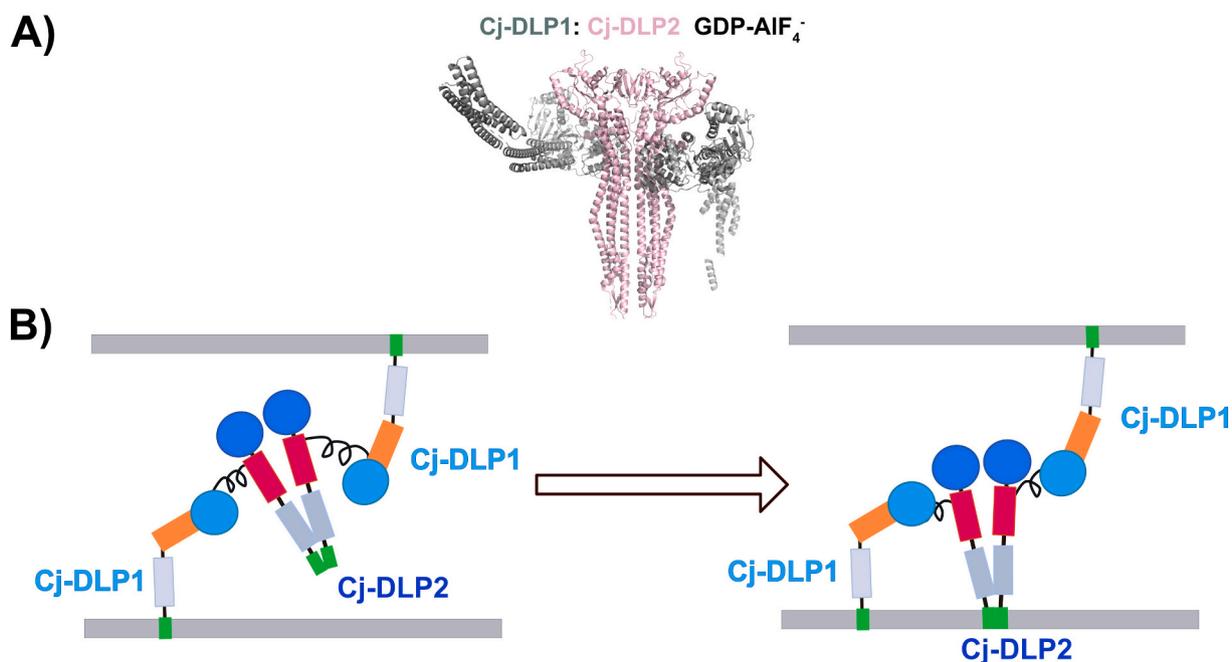


Fig. 7. (A) Crystal structure of Cj DLP1: Cj-DLP2 (2,2) tetramer in complex with GDP (PDB: 50WV) [63]. DLP1 is presented in gray while DLP2 in pink. Cj-DLP2 forms a central 'back-to-back' dimer which assembles on each side to Cj-DLP1 globular domain that nestles between their Neck and Trunk. (B) Schematic presentation of putative mechanism of recruitment of the tetramer to membranes. Tethering is dependent on Cj-DLP1 with Cj-DLP2 acting as an adaptor to extend the reach of bound Cj-DLP1 subunits. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

outer membranes remains elusive but recent developments have begun to open interesting perspectives.

Our first perception of mitofusins oligomers came from the crystal structure of the HR2 region of MFN1 (Fig. 9A) [100]. This isolated HR2 assembles into a dimeric anti parallel coiled coil that is 9.5 nm long (Fig. 9A-ii) [100]. Consistent with this, expression of truncated MFN1 lacking the GTPase domain induced formation of aggregated mitochondria with a uniform gap of 16 nm in a HR2 dependent manner [100]. This milestone study led to the hypothesis that MFNs promote mitochondrial tethering through anti-parallel coiled coil *trans*-MFN dimers in a GTP independent manner (Fig. 9A-iii). Yet, while nucleotide independent assemblies are documented for several DRPs such as OPA1/Mgm1 or DLP2 [63,87,88], this model excluded the involvement of the GTPase domain which was subsequently revealed as a key player in the oligomerization of most if not all DRPs [61,88,90]. Consistent with this, in vitro mitochondrial tethering by MFNs was shown to depend on GTP hydrolysis [108].

More recently, this apparent conundrum began to be circumvented through distinct studies [73,74,104,129]. Analysis of in vitro mitochondrial tethering reactions by cryo-electron tomography revealed distinct tethered and docked states of mitochondrial attachment [129]. In the presence of GMP-PNP, mitochondria tethered at a distance of 7–8 nm by repetitive globular assemblies containing Fzo1 [129]. In the presence of GTP, attached mitochondria were docked at a distance of 2–3 nm over extended area devoid of protein densities but surrounded and delimited at a distance of 6–8 nm by a ring-shaped macromolecular structure enriched in Fzo1 molecules and denominated as the mitochondrial docking complex (MDC) [129]. Importantly, outer membrane fusion was found to take place at one point of the MDC where membrane curvature was presumably most pronounced [129]. Taken together, this work hints at a model where GTP binding primes initial mitochondrial tethering by Fzo1, while hydrolysis brings outer membranes closer to each other. Successive repetition of this GTP binding/hydrolysis cycle around the initial site of apposition culminates in formation of the MDC before membrane fusion occurs at the MDC point of highest membrane curvature.

In the meantime, truncated versions of MFN1 and MFN2 which include the N-terminal portion connected to the extreme C-terminal end of mitofusins were purified and proved suitable for further characterization (Fig. 9B-i) [73,74,104,105]. The X-ray structures of these mini-MFN1 and mini-MFN2 constructs revealed a BDLP-like fold featuring a typical GTPase-domain followed by a four-helix bundle (HB1) which includes three helices from the GTPase domain and one helix from the HR2 domain (Fig. 9B) [73,74,104,105]. Mini-MFN1 (MFN1-MGD) structures were solved into two distinct closed and open conformations in the presence of GDP/BeF₃⁻ or GDP/AlF₄⁻ respectively (Fig. 9B-ii) [73,74]. The two structures differ in the orientation of the HB1 relative to the GTPase domain. These switches are again reminiscent of those seen for BDLP (Fig. 6A) and share resemblance with those reported for Atl (Fig. 4A). Moreover, mini-MFN1 was also found to dimerize in solution in the presence of different nucleotide analogs and to tether lipid vesicles [73,74]. Consequently, an Atl fusion model [72] was described for mitofusins during which G-domains mediate membrane attachment and moreover bring membranes in close apposition through GTP-dependent conformational changes of the formed *trans*-MFN G-dimers (Fig. 9B-iii). This model however raises a conflict with the initially HR2 based tethering model (Fig. 9A-iii) [100]. Interestingly, in Atl, GTP hydrolysis was deemed essential to dissociate existing *cis* G dimers prior to *trans* G-dimerization [72]. Similarly, one can thus not exclude that the GTPase domain may promote association of mitofusins in *cis*. However, compared to Atl, mitofusins harbor an additional predicted HB2 domain which provides an additional regulatory interface. Thus, the competing *cis/trans* G-oligomerization might not hold for mitofusins, and instead a sequential G-HB2 oligomerization is more likely to take place. The requirement of such macromolecular assembly for mitofusins is further supported by description of mitochondrial fission DRP assemblies during which G dimerization primes G-effector mediated oligomers [130].

Taking into consideration these distinct possibilities, a near full-length structural model of the yeast Fzo1 based on apo-BDLP has been used as a building block to predict distinct scenarios on putative 'sequential' oligomerization states of mitofusins (Fig. 10B) [131,132]. The GTPase and HB1 domains of this Fzo1 model are strikingly similar to

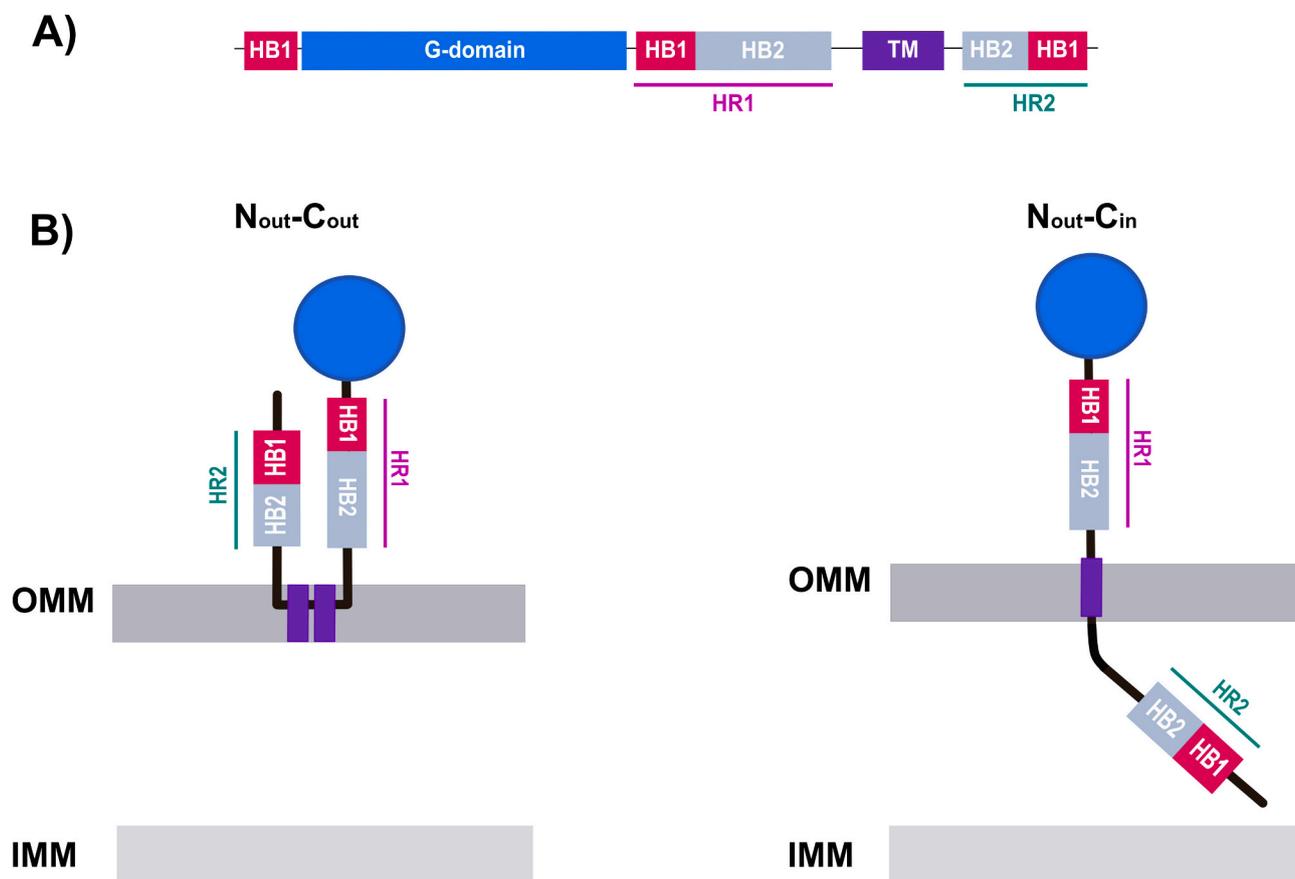


Fig. 8. (A) Molecular architecture of mitofusins. All mitofusins include an N-terminal GTPase domain (blue) and two C-terminal Heptad Repeat domains: HR1 (magenta line) and HR2 (green line), that sandwich a transmembrane region (purple). The yeast mitofusin includes an additional Heptad Repeat domain (HRN) located upstream the GTPase domain (not shown here). According to the crystal structure of truncated MFNs, the helices from the N-terminal part, the N-terminal helices of the HR1 domain and the extreme C-terminal half the HR2 domain assemble into a bundle denoted as HB1 and is represented here in red, while the complementary helices of the HR1 and HR2 domains are predicted to assemble into a second helix bundle denoted by HB2 and represented here in gray. (B) Possible topologies of mitofusins. (Left) A transmembrane region with two Transmembrane Domains (TMDs) gives mitofusin a topology in which the N- and C-terminal extremities are exposed to the cytoplasm (N_{out}-C_{out} topology) [99,114]. (Right) It was recently demonstrated that mitofusins from vertebrates could also include a single TMD, which keeps the N-terminal GTPase and HR1 domains in the cytoplasm but places the C-terminal HR2 domain in the mitochondrial intermembrane space (N_{out}-C_{in} topology) [116]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

those seen in the crystal structure of mini-MFNs [73,74,104,105,131]. Bringing further confidence in its accuracy, the model was experimentally validated through double swap mutations across predicted salt bridges that moreover corroborate with those seen in the mini-MFN1 crystal structure [74]. Consistent with the proposed role of HR2 in MFN1 [100], the Fzo1 model predicts an HR2 portion from the G-effector/HB2/Trunk domain of Fzo1 that is exposed to the solvent and may thus be available for *trans* tethering interactions [131]. Similarly, the HR1 portion from the Trunk is properly positioned to promote membrane destabilization, a property previously attributed to the HR1 of MFN1 and MFN2 [133]. Fzo1 building blocks were consequently employed to simulate their association on the same membrane to generate *cis*-dimers, which were subsequently assembled to predict formation of potential *trans* tetramers [132]. This led to the generation of 'head-to-head' and 'back-to-back' *cis*-models and resulting 'back-to-back' and 'head-to-head' *trans*-models. In these models, Fzo1 associates either in *cis* through its GTPase domain and in *trans* through its HR2 portion from the Trunk in an anti-parallel fashion or in *cis* through its HR2 portion from the Trunk in a parallel fashion and in *trans* through its GTPase domain [132]. Both *trans*-oligomerization systems conserved the ~8 nm distance between outer-membranes that was experimentally observed during mitochondrial tethering *in vitro* [129]. Both models also include the G-domain interface observed upon crystallization of

mini-MFNs dimers [74,104]. Interestingly, the 'back to back' *trans*-model is reminiscent of the antiparallel association seen with the isolated HR2 of MFN1 [100]. *Cis*-association of mitofusins may thus involve the GTPase domain rather than the HR2/Trunk domain as seen for BDLP [62].

The 'head-to-head' *cis*-model was further tested in a Coarse-Grained Molecular Dynamics (CG-MD) simulation to evaluate its stability and dynamics in a lipid environment (Fig. 10C) [134]. In this model, Ugo1 may bind to Fzo1 and favor *cis*-dimerization of the mitofusin through the activated GTPase domains which would be consistent with both the role of DRP co-factors in mitochondrial fission [130] and the proposed formation of Fzo1 *cis*-homodimers in a GTP and Ugo1 dependent manner [121]. *Cis* head-to-head dimers from opposing membranes would then engage in anti-parallel *trans*-interactions through the exposed hydrophobic spine from the HR2 located in the Trunk, resulting in the formation of tetramers that tether mitochondria (Fig. 10C) [134]. GTP hydrolysis within these tetramers would induce conformational switches in Fzo1 which would bring outer membranes closer together [134]. This Fzo1 tetramer model fits very well all requirements for formation of the MDC that may correspond to a macro-molecular complex of Fzo1 tetramers assembled as a Ring around the mitochondrial docked area.

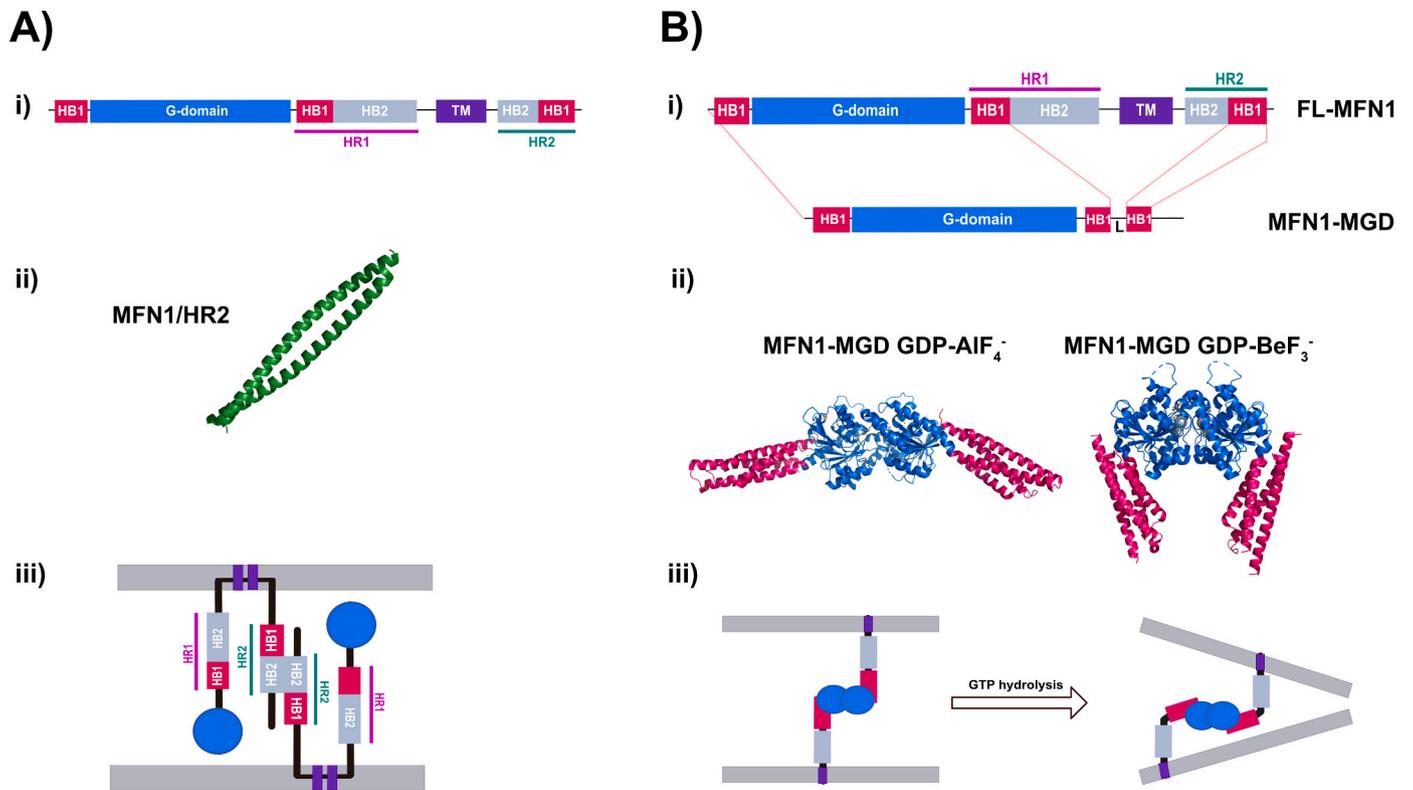


Fig. 9. A) (i) Molecular architecture of full-length mitofusin 1 (FL-MFN1). (ii) Crystal structure of the dimeric, antiparallel coiled coil MFN1/HR2 (PDB: 1T3J) [100]. (iii) Schematic model of GTP-independent *trans*-dimerization of MFNs. (B) (i) Molecular architecture of FL-MFN1 and schematic presentation of MFN1-MGD domain architecture; HB1 contains helices from the N-terminal part of MFN1, the HR1 domain and the extreme C-terminal half of MFN1. (ii): crystal structure of MFN1-MGD in complex to GDP-AIF₄⁻ (PDB: 5GOM) and GDP-BeF₃⁻ (PDB: 5YEW) affording open and closed conformations respectively [73,74]. (iii): A model for nucleotide-regulated switches in MFNs prior to tethering (left) and post tethering (right). Membrane attachment is mediated via trans G-dimers across OMM membranes, which upon GTP hydrolysis, transition toward a closed conformation bringing opposing membranes into close proximity.

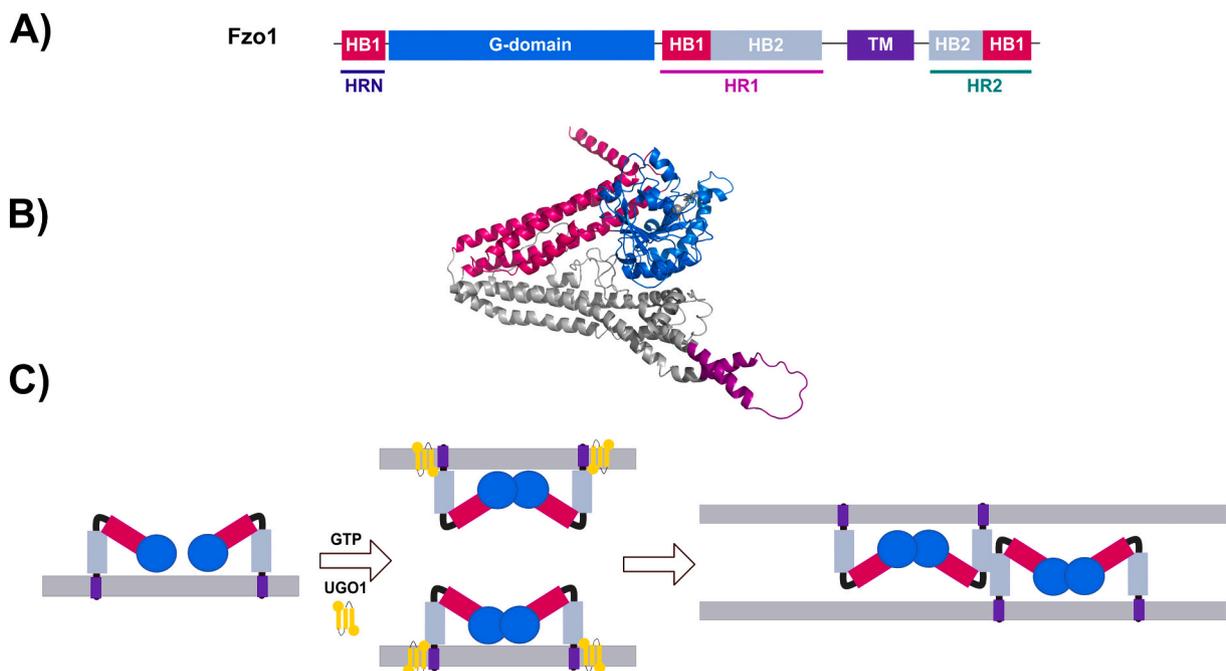


Fig. 10. (A) Molecular architecture of Fzo1 and schematic representation of Fzo1 domain architecture; (B) Fzo1 model showing the organization of the GTPase domain, HB1, HB2 and the transmembrane domain [130]. (C) Proposed Fzo1 model for sequential oligomerization in *cis* and *trans* assemblies. HRN is omitted from the model for clarity. Fzo1 form *cis* G dimers in a Ugo1 and GTP dependent manner. *Cis* dimers from opposing membranes form tetramers mediated by interaction anti-parallel interactions through HB2 [134].

6. Concluding remarks

While the functions and structures of DRPs continuously evolve, the specific case of mitofusins faces numerous outstanding questions that remain unresolved:

- What are the structures of full-length Fzo1, MFN1 and MFN2?
- What are the precise principles of cis- and trans-oligomerization of all mitofusins?
- What are the discrete functions of GTPase and HR domains in conformational switches, modes of interactions between mitofusins and their differential impact on membrane tethering and fusion?
- What is the possible involvement of established and potential co-factors in Fzo1 and MFNs oligomerization procedures?

Further experimental validation is required to unlock these distinct roadblocks. Fzo1, MFN1 and MFN2 full-length structures will ultimately reveal features that will boost the evolution of models currently employed to resolve the mechanistic understanding of yeast and mammalian mitochondrial fusion. Given the overall conservation of fission DRPs oligomerization properties, the accumulation of structures from fusion DRPs and their respective co-factors may in the long term unify the models that explain membranes anchoring and fusion by Dynamin-Related Proteins.

CRedit authorship contribution statement

MO wrote the paper and prepared the figs. MMC edited the whole manuscript and supervised the preparation of the figures.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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