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Biochimica et Biophysica Acta 1763 (2006) 482–489
BIOCHIMICA ET BIOPHYSICA ACTA
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Review

Molecular mechanism of mitochondrial membrane fusion

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Received 16 January 2006; accepted 14 February 2006
Available online 9 March 2006

Abstract

Mitochondrial fusion requires coordinated fusion of the outer and inner membranes. This process leads to exchange of contents, controls the shape of mitochondria, and is important for mitochondrial function. Two types of mitochondrial GTPases are essential for mitochondrial fusion. On the outer membrane, the *fuzzy onions*/mitofusin proteins form complexes *in trans* that mediate homotypic physical interactions between adjacent mitochondria and are likely directly involved in outer membrane fusion. Associated with the inner membrane, the OPA1 dynamin-family GTPase maintains membrane structure and is a good candidate for mediating inner membrane fusion. In yeast, Ugo1p binds to both of these GTPases to form a fusion complex, although a related protein has yet to be found in mammals. An understanding of the molecular mechanism of fusion may have implications for Charcot–Marie–Tooth subtype 2A and autosomal dominant optic atrophy, neurodegenerative diseases caused by mutations in *Mfn2* and *OPA1*.

Keywords: Membrane fusion; Mitochondrial dynamics; Mitochondrial fusion; GTPase; Organelles

1. Introduction

It has been known for decades that mitochondria have plasticity of form and undergo fusion and fission [1], but only recently has there been progress in elucidating the molecular basis of these processes. A breakthrough was the identification of the *fuzzy onions* gene product (Fzo) in the fusion of mitochondria during *Drosophila* sperm differentiation [2]. Fzo is the founding member of a family of mitochondrial outer membrane GTPases essential for mitochondrial fusion from yeast to mammals [2–4]. Subsequent genetic studies have identified additional components of the fusion and fission machinery.

The balance between fusion and fission plays a central role in controlling mitochondrial morphology. In the absence of fusion, mitochondria fragment into small spheres due to ongoing fission [3–6]. Beyond its role in morphology, mitochondrial fusion is required for mitochondrial function. Mammalian cells lacking mitochondrial fusion grow slowly due to low respiratory capacity [5]. Mice deficient in mitochondrial fusion die in midgestation [4]. Moreover, mutations in components of the fusion pathway have been implicated in human neurodegenerative diseases: *Mfn2* in the peripheral neuropathy Charcot–Marie–Tooth Disease subtype 2A and *OPA1* in autosomal dominant optic atrophy [7–11].

In this review, we discuss our current understanding of how mitochondrial membranes fuse. To begin, we outline how lipid bilayers fuse in the two best-studied experimental systems – virus-mediated fusion and vesicle fusion – in order to highlight general principles of membrane trafficking that may be applicable to mitochondrial fusion.

2. Virus/host membrane fusion

In order for an enveloped virus to gain access to the cellular compartment, it must direct fusion between the viral membrane and the host cell membrane. This reaction is mediated by virally encoded transmembrane glycoproteins embedded in the viral envelope. Specificity of fusion is provided by the binding of the viral glycoprotein to specific cell surface receptors on the host cell. Class I viral fusion proteins contain a short hydrophobic helix termed the fusion peptide that is crucial to the fusion reaction. On native virions (the prefusogenic state), the fusion peptide is buried in a

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doi:10.1016/j.bbamcr.2006.02.003

hydrophobic pocket in the glycoprotein interior [12,13]. Importantly, this native structure is metastable. That is, it is stably folded but can be triggered to undergo a conformational change to a more thermodynamically stable structure. Contact with host receptors (in the case of HIV-1 gp41) or a pH change following transport to the endosome (in the case of influenza HA2) triggers a dramatic structural transition that results in extension and insertion of the fusion peptide into the host membrane. In this extended conformation, the transmembrane fusion protein now bridges the viral and host membrane, although a gap remains between the membranes [12]. This conformation is a transient intermediate, and the fusion protein subsequently undergoes a second structural transition to snap the fusion peptide and transmembrane regions together. This final structure is highly stable and results from the pairing of two regions containing hydrophobic heptad repeats, sequence motifs that form coiled-coil-like structures [12]. In this fusogenic conformation, the viral and host membranes are forced into close apposition. Therefore, the charge repulsion between the lipid bilayers is overcome by coupling membrane apposition with the formation of a highly stable helical bundle. This sequence of events appears to occur for a broad range of Class 1 viral fusion proteins [12,13].

3. SNARE-mediated intracellular fusion

Most intracellular membrane fusion events utilize a very similar mechanism. Rab GTPases, Rab effector molecules and SNAREs are the core components of the membrane fusion machinery [14,15]. Their segregation patterns are largely responsible for marking membranes that are capable of fusing with each other. The interaction of Rab GTPases and Rab effectors in *trans* provides the initial tethering between membranes and helps ensure that only the correct pairs of membranes can proceed to fusion. At this stage, the membranes are reversibly associated but still separated by a considerable gap.

After this tethering stage, membrane fusion itself is likely to be mediated by SNARE proteins residing in the two membranes. SNAREs are membrane-associated proteins containing a loosely conserved ~60 residue SNARE motif that contains hydrophobic heptad repeats. SNAREs can be engineered to fuse liposomes [16] and cells [17] and are necessary in most intracellular membrane fusion systems, although there are exceptions [15]. Prior to fusion, distinct Q- and R-SNAREs are segregated between the two membranes and "primed" for fusion. Following Rab-mediated tethering, the Q-SNAREs interact specifically with their cognate R-SNARE partners in *trans*. The specificity of SNARE pairing further ensures the fidelity of the fusion reaction [18,19]. The *trans* SNARE complex adopts an extremely stable structure, in which four SNARE motifs form a four helix bundle to draw the two membranes together [20]. Similar to the viral fusion proteins, the energetic benefit of forming the *trans* SNARE structure is coupled to overcoming the charge repulsion between the membranes.

4. General considerations of mitochondrial membrane fusion

There are several important characteristics of mitochondria that make their fusion mechanism particularly intriguing. First, unlike almost all other intracellular fusion events, neither SNAREs nor the AAA-ATPase NSF have been implicated in the mitochondrial fusion reaction [21]. Indeed, the three known mitochondrial fusion molecules appear solely dedicated to mitochondrial fusion, suggesting the machinery evolved independently and is uniquely tailored for this organelle. Interestingly, a different machinery may exist in plants, because there are no identifiable homologs of the animal genes involved in fusion [22]. Second, mitochondria have an outer and inner membrane, with a membrane potential across the latter. Therefore, the fusion of four sets of lipid bilayers must be coordinated. Third, unlike viral fusion and most SNARE-mediated fusion, mitochondrial fusion is homotypic. This implies a symmetry in the distribution of molecules between the adjacent membranes and is undoubtedly reflected in their fusion mechanism. Finally, although the regulation of mitochondrial fusion is not understood, it is likely to be influenced by cellular energetic demands, apoptotic stimuli and developmental cues. Taken together, these characteristics suggest that mitochondria fuse through a novel mechanism that reflects their unique endosymbiont origin and double membrane architecture.

In spite of these unique features, mitochondrial fusion likely has some general features in common with virus-mediated and SNARE-mediated membrane fusion. First, the specificity of membrane fusion is likely determined by the formation of specific protein complexes formed in *trans* between the fusing membranes. As detailed below, mitofusin complexes on the outer membrane may provide the specificity for homotypic inter-mitochondrial interactions. Second, conformational changes in *trans* protein complexes will likely provide the energy necessary to appose two negatively charged lipid bilayers. Again, mitofusins may be critical for this process.

A number of experimental systems have been developed to study mitochondrial fusion. In some studies, tubulation of mitochondria is interpreted as evidence for fusion. However, the assessment of mitochondrial fusion by morphology alone is risky, because factors other than fusion, such as loss of fission, can influence the tubulation of mitochondria. Therefore, direct in vivo assays for fusion are the foundation for most of our knowledge of the genetic and energetic requirements for mitochondrial fusion. In yeast, fusion between differentially labeled mitochondria is scored following mating of *a* and α cells [21]. In mammalian tissue culture, cells with distinctly labeled mitochondria are co-plated and fused by treatment with polyethylene glycol (PEG). The resulting cell hybrids are scored for the intermixing of mitochondrial markers [4,23,24]. Alternatively, the diffusion of a photoactivated mitochondrial GFP can be used to quantitate mitochondrial fusion [25]. Recently, the development of an in vitro mitochondrial fusion assay has opened the door to integration of genetic and biochemical approaches [26].

5. The players

Genetic strategies have identified three core components of the fusion pathway in yeast (Fig. 1). Two of these genes, Fzo1p and Mgm1p, encode large mitochondrial GTPases. The third, Ugo1p, resides in the mitochondrial outer membrane and interacts with both Fzo1p and Mgm1p. There are two mammalian homologs of *FZO1*, termed mitofusins (*Mfn1* and *Mfn2*) and one homolog of *MGM1*, *OPA1*. A mammalian homolog of *UGO1* has not been identified. In yeast, two additional components, Mdm30p and Pcp1p/Rbd1p, regulate the activity of Fzo1p and Mgm1p, respectively.

6. FZO family

6.1. FZO family members are required for mitochondrial fusion

Fzo family members are the best candidates for molecules that directly mediate mitochondrial fusion. *Fzo/Mfn* mutations in yeast, flies and mammals completely abolish mitochondrial fusion, indicating they play essential, conserved roles in fusion [2,3,5]. In *fzo1Δ* yeast, even mitochondria that are adjacent to one another are unable to fuse, suggesting Fzo1p acts at a late step in the fusion pathway [3]. Similarly, *Mfn*-null embryonic fibroblast cell lines (lacking both *Mfn1* and *Mfn2*) have severely fragmented mitochondrial morphology and display no fusion in the PEG mitochondrial fusion assay [5].

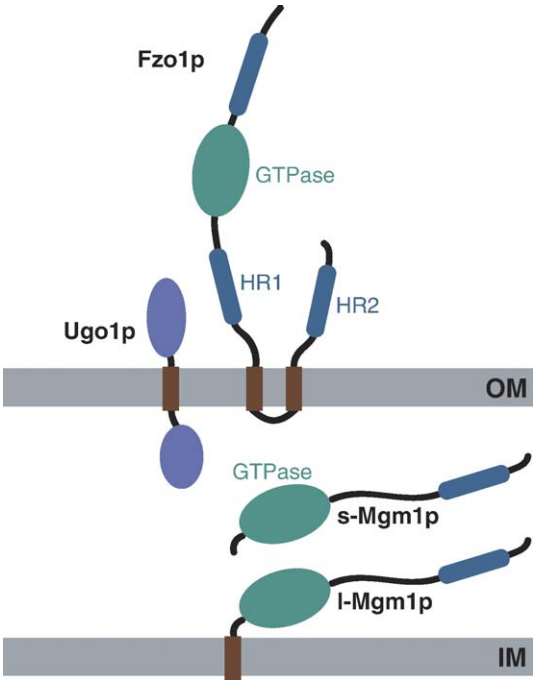

Fig. 1. The mitochondrial fusion complex in yeast. Fzo1p spans the outer membrane twice, placing the GTPase domain (green ellipse) and three heptad repeats (blue rods) in position to mediate important steps during fusion. The long and short isoforms of the dynamin-related GTPase Mgm1p are located in the intermembrane space and differ in the presence of an N-terminal transmembrane region. Ugo1p interacts with both Fzo1p and Mgm1p, and may help coordinate their activities. The mitofusins and OPA1 are the mammalian orthologs of *FZO1* and *MGM1*. Mitofusins contain HR1 and HR2 but lack the most N-terminal hydrophobic heptad repeat. No mammalian ortholog of *UGO1* has been identified.

Studies of embryonic fibroblast cell lines derived from *Mfn1* mutant and *Mfn2* mutant mice indicate that Mfn1 and Mfn2 are partially redundant molecules [4]. In contrast to *Mfn*-null cells, *Mfn1*-null cells and *Mfn2*-null cells retain low levels of mitochondrial fusion [4,5]. Both of these mutant cell lines also have fragmented mitochondrial morphologies. *Mfn1*-null cells have mitochondria that are extremely short and rod-shaped, while *Mfn2*-null cells have mitochondria that range from small fragments to large spheres and short tubules. Significantly, a highly tubular mitochondrial population can be restored in either *Mfn1*-null, *Mfn2*-null, or *Mfn*-null cells by the over-expression of either Mfn1 or Mfn2 [4,32]. This morphological rescue is accompanied by restoration of full fusion activity. These data indicate that a single mitofusin is sufficient for mitochondrial fusion and that the defects observed in the single mutant cell lines result from the reduced level of total mitofusin expression rather than the specific requirement for either molecule.

6.2. The architecture of Fzo family members

Fzo family members encode large transmembrane GTPases that are distributed uniformly across the mitochondrial outer membrane. The transmembrane region spans the outer membrane twice, placing the N- and C-terminal portions in the cytosol where they are in position to mediate important steps during fusion (Fig. 1) [3,6,27].

Fzo family members share an N-terminal GTPase domain that includes the canonical G1–G4 motifs. Mutations designed to block GTP nucleotide binding or hydrolysis completely block fusion [2–4]. However, despite its required role in fusion, there is little data that indicates how GTPase activity contributes to the fusion reaction. The GTPase domain may function in a regulatory/signaling capacity similar to that of Rab GTPases during SNARE-mediated fusion. In this model, the nucleotide state of Fzo/Mfn would regulate the recruitment or activity of other factors during fusion. Alternatively, Fzo/Mfn could function in a manner similar to dynamin GTPases, and couple GTP hydrolysis to a mechanochemical activity such as membrane deformation or close membrane apposition. Indeed, it has been suggested that Fzo/Mfn is a dynamin family member on the basis of its large size, membrane association and ability to oligomerize [28].

Fzo family members contain two heptad repeat regions, HR1 and HR2, situated on either side of the transmembrane region. The yeast homolog encodes a third heptad repeat region N-terminal of the GTPase domain that is not found in the mammalian mitofusins. As discussed previously, hydrophobic heptad repeats are predicted to form coiled-coil-like structures and play critical functions in the fusion mechanism of both SNAREs and viral fusion proteins. Given their location proximal to the transmembrane region, it is tempting to speculate that HR1 and/or HR2 might form helical fusogenic structures analogous to those formed by SNAREs or viral glycoproteins.

Like dynamins, Fzo/Mfn molecules are capable of complex intermolecular interactions. Co-immunoprecipitation studies have demonstrated that the mitofusins can interact to form three distinct molecular complexes: Mfn1 homotypic complexes, Mfn2 homotypic complexes and Mfn1/Mfn2 heterotypic complexes [4,29]. Coexpression of a mitochondrially localized C-terminal construct of Mfn2 can sequester an otherwise cytoplasmic N-terminal Mfn2 construct [30]. This recruitment is dependent on the presence of both HR1 and HR2 and suggests they may interact with each other [30]. Additionally, the C-terminus of Mfn2, lacking the transmembrane region can be immunoprecipitated with the N-terminus of Mfn2 in a reaction which depends on the GTPase domain and HR1 [31]. The stoichiometry of these complexes is unknown and direct interactions have not been demonstrated.

6.3. Fzo/Mfn forms a trans complex

During both viral and SNARE-mediated fusion, the formation of specific *trans* complexes is critical for ensuring specificity and for mediating membrane apposition. For these reasons, it is important to understand what *trans* complexes form between adjacent mitochondria during mitochondrial fusion. Because mitochondrial fusion is homotypic, specificity could be achieved by an interaction between the same protein on adjacent mitochondria. Several lines of evidence implicate Fzo/Mfn in the formation of a *trans* complex. Mfn-null mitochondria fail to fuse with wild-type mitochondria, indicating that mitofusins are required on adjacent mitochondria [32]. In addition, *Mfn1*-null mitochondria can fuse to *Mfn2*-null mitochondria, suggesting that a *trans* Mfn1/Mfn2 complex is also fusion-competent [5]. Finally, mitochondrial fusion in vitro requires functional *FZO1* on both mitochondria, supporting its role in mediating a *trans* complex [26].

The C-terminal HR2 domain of Mfn1 appears to be important for formation of a *trans* complex. HR2 forms a dimeric, anti-parallel coiled coil that is 95 Å long [32]. In contrast to the fusogenic states of viral fusion proteins and SNAREs, the anti-parallel HR2 dimer positions the transmembrane domains on opposite ends of the helical bundle. Therefore, formation of this structure between Mfn1 molecules on adjacent mitochondria would tether the membranes together, but not close enough to directly mediate fusion. Expression of a truncated Mfn1 lacking the GTPase domain results in severe mitochondrial clumping [32]. EM images of these clumped mitochondria reveal tight and uniform spacing compatible with the length of the dimeric HR2 coiled coil. Formation of these structures depends on the HR2 structure and may represent a tethered intermediate in the fusion pathway [32]. Because these tethered intermediates are unable to progress to membrane fusion, the GTPase domain likely acts downstream of mitochondrial tethering. A *trans* Mfn1 complex has also been identified in vitro by immunoprecipitation [33].

6.4. MDM30 controls Fzo1p levels

MDM30 was identified in a visual screen for non-essential yeast genes required to maintain normal mitochondrial morphology [34]. Mdm30p is a member of the F-box family of proteins, which target substrates to the SCF (Skp1p/Cdc53p/F-box) ubiquitin ligase complex. Ubiquitinated substrates are then degraded by the 26S proteasome [35]. *mdm30Δ* cells have elevated Fzo1p levels that result in fragmentation and aggregation of the mitochondrial reticulum [36]. In the zygotic fusion assay, *mdm30Δ* cells have no fusion activity, but *mdm30Δ dnm1Δ* double mutants (*DNM1* is required for mitochondrial fission) retain some activity, indicating *MDM30* is important but not strictly required for mitochondrial fusion [36].

In the simplest model, Mdm30p could regulate the turnover rate and the steady-state levels of Fzo1p by directly targeting Fzo1p for degradation. Alternatively, it has been suggested that Mdm30p may degrade non-productive Fzo1p fusion intermediates, whose accumulation in *mdm30Δ* yeast could inhibit fusion [36]. An important step in testing these models will be to determine the direct targets of Mdm30p, and specifically if Fzo1p is one of them. It has not been possible to demonstrate the presence of ubiquitin conjugated Fzo1p molecules, and it remains possible that the elevated Fzo1p expression levels in *mdm30Δ* yeast is an indirect effect [36]. In addition to Mdm30p-dependent turnover of Fzo1p, it has also been noted that Fzo1p degradation following mating factor treatment is Mdm30p-independent [37]. Therefore, at least two distinct modes of Fzo1p turnover exist.

7. MGM1/OPA1

7.1. The role of MGM1/OPA1 in mitochondrial fusion

Mgm1p/OPA1 is a member of the dynamin-related protein (DRP) subfamily. DRPs and classical dynamins all encode GTPase, middle, and GTPase effector (GED) domains. Unlike dynamins, DRPs lack a pleckstrin homology and a proline-rich domain [28]. Several dynamin family members have well-characterized roles in membrane fission in diverse cellular contexts including endocytosis, vesicular trafficking, and mitochondrial division. During endocytosis, dynamin assembles into a collar around the neck of the invaginating vesicle. GTP hydrolysis is thought to stimulate constriction or elongation of the collar, leading to membrane scission. It has not been determined if Mgm1p shares the membrane-constricting activity of the other dynamin family members, and it is unclear how such an activity would contribute to mitochondrial fusion.

The requirement of Mgm1p for mitochondrial fusion in yeast has been clearly established. *mgm1Δ* cells display no mitochondrial fusion, even when the mitochondria are in close contact, indicating that Mgm1p, like Fzo1p, is required at a late step in the fusion pathway [38,39]. There has been significant confusion over the function of OPA1 because, paradoxically, both over-expression and knock-down of OPA1

result in fragmentation of the mitochondrial network [5,40–42]. In one experimental system, OPA1 expression leads to increased tubulation [42]. While the over-expression phenotype remains to be resolved, it is clear that mitochondrial fragmentation in OPA1 knock-down cells results from a block in membrane fusion activity [5,42].

In addition to the defects in fusion, cells lacking OPA1 have highly disorganized cristae [40,41]. Similarly, *mgm1Δ* yeast have dramatically swollen and poorly involuted cristae [38]. These observations raise the issue of whether the fusion defects in Mgm1p/OPA1-deficient cells might be secondary to disorganization of the mitochondrial inner membrane. However, the cristae defects in *mgm1Δ* cells are largely suppressed in *dnm1Δ mgm1Δ* cells, but these cells nevertheless are completely defective for mitochondrial fusion [38,39]. Taken together, these observations implicate Mgm1p/OPA1 directly in the fusion reaction.

7.2. Maturation of Mgm1p

Correct processing of Mgm1p is critical to its function in mitochondrial dynamics. In yeast, proteolytic processing results in a long and a short isoform, l-Mgm1p and s-Mgm1p. Unprocessed Mgm1p contains an N-terminal mitochondrial targeting sequence (MTS) followed by an extended hydrophobic region. After translation in the cytosol, Mgm1p is targeted to the mitochondrial inner membrane by the MTS [43]. The MTS is inserted through the inner membrane and into the matrix, where it is cleaved by the mitochondrial processing peptidase (MPP), resulting in l-Mgm1p [43]. l-Mgm1p is believed to be anchored in the inner membrane through the N-terminal hydrophobic domain, leaving the rest of the protein facing the inter-membrane space.

Mgm1p can be also be processed by the rhomboid-like protease Rbd1p/Pcp1p to yield s-Mgm1p [43–46]. Rhomboids are a functionally conserved family of intramembrane proteases best characterized for their processing of epidermal growth factor receptor ligands in *Drosophila* [47]. s-Mgm1p remains membrane associated in the IMS, although whether s-Mgm1p is associated with the IMS face of the IM or OM is unclear [43]. It has been proposed that sorting of Mgm1p in the inner membrane is responsible for the production of l-Mgm1p versus s-Mgm1p [46]. During cleavage of the MTS, the N-terminus of Mgm1p lies within the translocon of the inner membrane (TIM). If Mgm1p exits laterally out of the TIM complex, l-Mgm1p is produced. If Mgm1p exit is delayed, further insertion of the hydrophobic domain allows processing by Rbd1p/Pcp1p to produce s-Mgm1p. This model is consistent with the observation that l-Mgm1p and s-Mgm1p do not have a precursor–product relationship [48].

rbd1/pcp1Δ cells produce only l-Mgm1p [43–45]. These cells display severely fragmented mitochondrial morphology, indicating l-Mgm1p is not sufficient to support normal levels of mitochondrial fusion. Assessing the activity of s-Mgm1p is more difficult because it lacks a MTS. However, s-Mgm1p targeted to mitochondria by fusion of a heterologous N-terminal MTS fails to complement the fusion defect in *mgm1Δ* cells [43]. The ratio of the two isoforms is critical for fusion because mutations which cause differences in the ratio of the two isoforms cause defects in mitochondrial morphology [43–46]. Allelic complementation between temperature-sensitive *mgm1* mutants indicate Mgm1p is multimeric, and it would be interesting to know if the two isoforms interact with each other, or if they form distinct complexes [39].

OPA1 in mammalian cells is also localized to the mitochondrial inter-membrane space and is peripherally associated with the inner membrane [40,49]. The mammalian homolog of Rbd1p/Pcp1p, PARL, is indeed localized to mitochondria. However, its putative role in OPA1 processing remains to be confirmed. An additional complication is that extensive alternative splicing results in 8 mRNA isoforms [8,50].

7.3. UGO1

FZO1 and *MGM1* are just two of the ~340 genes required for respiration in *S. cerevisiae*. They are unique, however, because their respiration defect can be suppressed by mutations in the mitochondrial fission pathway [51–54]. This property of mitochondrial fusion mutants enabled the identification of *UGO1* as a third component of the fusion pathway [55]. Ugo1p is a 58-kDa protein embedded in the mitochondrial outer membrane, with the N-terminal region facing the cytosol and the C-terminal region facing the inter-membrane space [55]. Ugo1p contains two energy transfer motifs found in mitochondrial carrier proteins. However, no Ugo1p homologs have been identified outside of fungi, suggesting that its role in mitochondrial fusion is not conserved.

Ugo1p, Fzo1p and Mgm1p assemble into a fusion complex. The N-terminal cytoplasmic domain of Ugo1p binds Fzo1p near its transmembrane region, and the C-terminal half of Ugo1p binds Mgm1p in the inter-membrane space [56]. Indeed, Ugo1p is required for the formation of a Fzo1p/Mgm1p complex [39,56]. Interestingly, the GTPase activities of Fzo1p and Mgm1p are not required for binding to Ugo1p, suggesting that Ugo1p binding does not require the GTPase cycles of either protein [56]. Based on these data, Ugo1p may provide a scaffold for the assembly of a fusion complex that spans the outer and inner membranes, and could provide a link that coordinates their fusion. The requirement for this interaction would explain why Mgm1p is required for outer membrane fusion despite its inter-membrane space localization [38].

8. Ergosterol

Ergosterol is a membrane-associated sterol with a recently established role in vacuolar and peroxisomal fusion. Interestingly, several lipids including ergosterol, PI(3)P, PI(4,5)P_2, and diacylglycerol (DAG) organize specific vacuolar membrane subdomains where fusion proteins are concentrated [57]. Disruption of ergosterol biosynthesis leads to vacuolar fusion

defects both in vitro and in vivo [58]. Similarly, ergosterol, PI(3) and PI(4,5)P_2 inhibitors block peroxisomal fusion in vitro by preventing fusion proteins from dynamically partitioning in membrane subdomains [59].

A striking conclusion from two recent genome wide screens for morphology defects in yeast is the identification of severe mitochondrial morphology defects in mutants of the ergosterol biosynthesis pathway. These mutants all possess fragmented or aggregated mitochondria, suggesting a fusion defect [34,63]. Although a direct role in fusion has not been established with either the zygotic or in vitro mitochondrial fusion assay, an intriguing possibility is that ergosterol is necessary to organizing fusion molecules into subdomains in the outer membrane. Both Fzo1p and Ugo1p are uniformly distributed across the outer membrane, so either other unknown components or fusion-active subpopulations of these proteins may be concentrated into ergosterol-rich domains.

9. How are outer and inner membrane fusion coordinated?

The double membrane architecture of mitochondria means that fusion necessarily entails merging four membranes into two membranes. Time-lapse imaging studies in vivo indicate that outer and inner mitochondrial membrane fusion are tightly coordinated. Manipulation of mitochondrial fusion in vitro, however, has distinguished outer membrane and inner membrane fusion as two mechanistically distinct processes [26]. Under limiting GTP concentrations, mitochondria in vitro will fuse their outer membranes, but not inner membranes. Supplementation with a GTP regenerating system allows for subsequent inner membrane fusion [26].

Similarly, outer membrane fusion can be distinguished from inner membrane fusion in mammalian cells. Dissipation of mitochondrial membrane potential has been reported to block mitochondrial fusion [24,60,61]. Surprisingly, this effect is specific for inner membrane fusion. Treatment of human 143B cells with an H+ ionophore (CCCP) or a K+ ionophore (valinomycin) blocked inner membrane but not outer membrane fusion [62]. In contrast, CCCP treatment in vitro nearly completely blocks outer membrane fusion in yeast [26]. It will be interesting to determine whether the membrane fusion defects observed with depolarized mitochondria are due to a direct mechanistic role of membrane potential during the fusion process, or secondary to inner membrane ultrastructural defects observed following depolarization.

The requirement for high levels of GTP for inner membrane fusion in vitro implicates a GTPase in inner membrane fusion. A likely candidate is the inter-membrane space GTPase Mgm1p. As discussed above, because Mgm1p is necessary for outer membrane fusion in vivo, it has not been possible to address its role specifically in inner membrane fusion [38]. Because the in vitro mitochondrial fusion assay allows distinction between outer membrane and inner membrane fusion, it should be useful in addressing this issue through the use of temperature-sensitive alleles of *MGM1*.

10. Perspectives

Based on the studies discussed, the outlines of a model for mitochondrial fusion can be proposed to motivate future studies. It seems likely that Fzo1p/Mfns and Mgm1p/OPA1 are core components of the fusion machinery. Fzo1p/Mfn on the outer membrane appears to mediate homotypic interactions between mitochondria. They play a direct role in at least tethering mitochondria to each other, and may have subsequent roles in membrane merger. Based on its location, Mgm1p/OPA1 is the best candidate for mediating inner membrane fusion. It is unclear how its putative dynamin-like properties would contribute to fusion. One possibility is that OPA1 is important in tubulation of the inner membrane during the fusion process. In yeast, Ugo1p may help to coordinate outer membrane fusion by Fzo1p with inner membrane fusion by Mgm1p, but nothing is known about its possible counterpart in mammalian cells.

A clear priority for the future is the identification of new components of the fusion pathway. Genetic screens in yeast have been very successful in identifying much of the core machinery, and these can now be complemented with biochemical approaches. In the near future, it may be possible to identify the core mitochondrial fusion machinery and to determine how it mediates apposition and fusion of mitochondrial membranes. To achieve this goal, a combination of structural information and in vitro reconstitution using purified components will be necessary.

Acknowledgments

Work in D. Chan's laboratory is funded by the NIH (GM062967), the Muscular Dystrophy Association, and the United Mitochondrial Disease Foundation. D. Chan is a Bren Scholar.

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