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Review

Determinants and outcomes of mitochondrial dynamics

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SUMMARY

Mitochondria are not only central organelles in metabolism and energy conversion but are also platforms for cellular signaling cascades. Classically, the shape and ultrastructure of mitochondria were depicted as static. The discovery of morphological transitions during cell death and of conserved genes controlling mitochondrial fusion and fission contributed to establishing the concept that mitochondrial morphology and ultrastructure are dynamically regulated by mitochondria-shaping proteins. These finely tuned, dynamic changes in mitochondrial shape can in turn control mitochondrial function, and their alterations in human diseases suggest that this space can be explored for drug discovery. Here, we review the basic tenets and molecular mechanisms of mitochondrial morphology and ultrastructure, describing how they can coordinately define mitochondrial function.

UNDERSTANDING MITOCHONDRIAL DYNAMICS

Classically, reviews on mitochondria start by describing these organelles as the "powerhouses" of the cell, which are in charge of cellular respiration, oxidation of NADH and $FADH_2$, and ATP biosynthesis. Although such statements are undoubtedly true and represent central tenets of bioenergetics, additional mitochondrial functions beyond metabolism have contributed to making the term "mitochondria" more popular in medical literature than other key organelles and subcellular domains, including the nucleus.

Mitochondria display a complex morphology. The inner mitochondrial membrane (IMM) and outer mitochondrial membrane (OMM) delimit the intermembrane space (IMS). The mitochondrial matrix is enclosed by the IMM, which is folded into invaginations called cristae. A key feature of mitochondria is their dynamic nature. The locution "mitochondrial dynamics" collectively describes the ability of mitochondria to (1) be transported along the cytoskeleton, (2) interact with other subcellular structures, and (3) shape their IMM and OMM through fusion and fission events. Fusion represents the process of merging two or more mitochondria to generate a larger organelle. Fission is the process of constricting and severing one mitochondrion to generate smaller organelles. Fusion and fission cycles are controlled by a set of "mitochondria-shaping" proteins that we will describe in detail below and are central to every aspect of mitochondrial dynamics. Moreover, these proteins are embedded in a growing list of cellular signaling cascades that utilize mitochondria as signaling hubs. Fusion and fission are also required for mitochondrial biogenesis and for mitophagy, the organelle degradation by autophagy. Ultimately, fusion and fission regulate virtually every facet of mitochondrial physiology[1,2] (Figure 1). It is therefore not surprising that mutations in key fusion and fission genes result in genetic diseases and that mitochondrial dynamics appear altered in numerous sporadic disorders, substantiating the importance of mitochondrial dynamics for cellular function.

In this review, we provide an account of the molecular mechanisms of mitochondrial fusion and fission and of cristae biogenesis and remodeling. We also describe how these mechanisms impinge on mitochondrial physiology. We refer the reader to other reviews that detail the processes of mitochondrial transport and interaction with other organelles (for example, Vanhauwaert et al.[8] and Giacomello et al.[9]).

The discovery of mitochondrial morphology genes

The first core pro-fusion molecular player was discovered in 1997 in *D. melanogaster*. In spermatids lacking the gene *fuzzy onion* (*FZO1*), mitochondria failed to form the *nebenkern*, the giant mitochondrion occupying the spermatid tail, leading to the appearance of numerous smaller mitochondria. Of note, fzo1 GTPase activity was essential for the process of mitochondrial fusion, establishing the role of guanosine triphosphate (GTP) hydrolysis in mitochondrial morphology regulation.[10] The yeast homolog, *Fzo1p*, was discovered one year later in *S. cerevisiae* as an integral membrane protein with a GTPase domain facing the cytoplasm. Fzo1p was required for fusion during mating and for mitochondrial respiration, growth, and mtDNA maintenance.[11,12] Finally, two mammalian Fzo homologs named mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2) were identified.

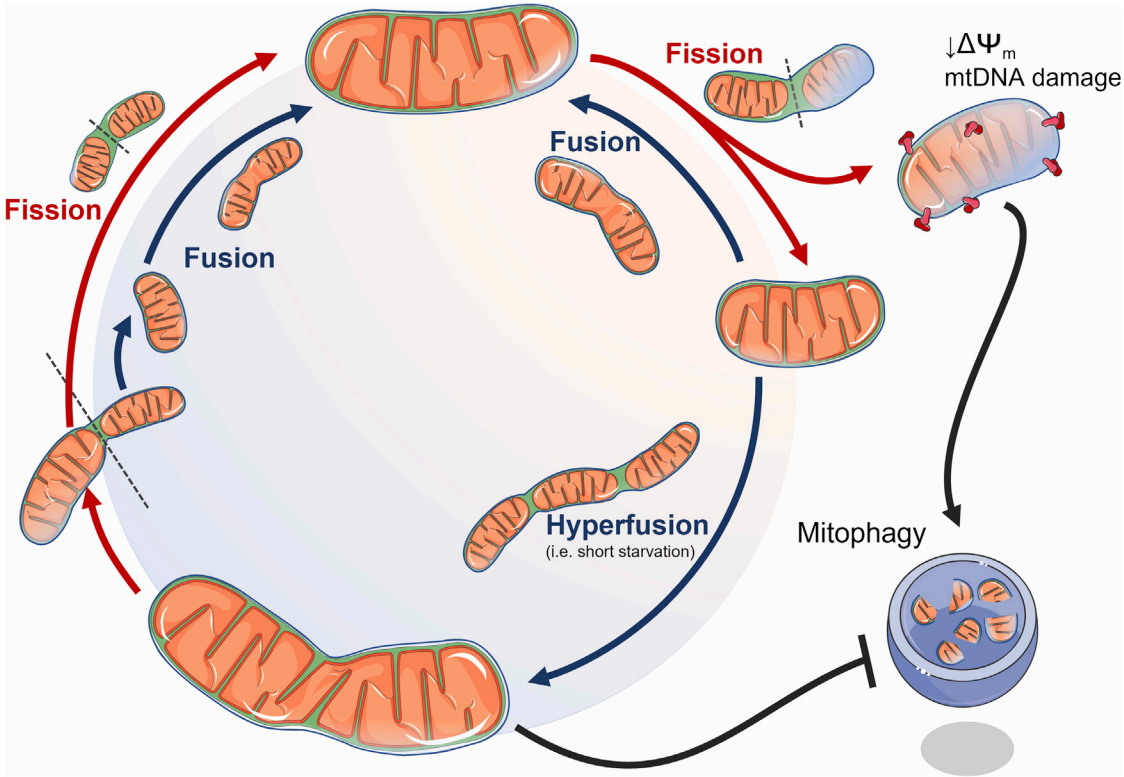

Figure 1. Mitochondrial fusion and fission

A cyclic process: starting from the top, a mitochondrion can undergo fission (red lines) under certain conditions such as mtDNA damage or depolarization. This process is reversible, and one of the functional daughter mitochondria can fuse (blue lines) to a new one to recompose a larger organelle. Fission occurs with the severing of the inner and outer membranes (dotted line), and damaged components from the native organelle can be sorted toward segregated, dysfunctional mitochondria, subsequently targeted for mitophagy. This process can be transiently prevented by a reduced fission or a hyperfusion of several mitochondria that results in elongated organelles,[3,4,5] as observed under mild starvation. The resulting elongated mitochondria (bottom) harbor more packed cristae and conserved bioenergetics[6,7] and are excluded from mitophagy.[3] Once the insult is resolved, hyperfused mitochondria undergo fission and fusion cycles to stabilize the native network.

These two proteins display a 77% degree of similarity, harbor a GTPase domain, and induce mitochondrial fusion and perinuclear clustering when overexpressed.[13]

The discovery of the inner membrane proteins mitochondrial genome maintenance 1 (Mgm1p) in *S. cerevisiae* and its homolog Msp1p in *S. pombe* added two key genes to the mitochondrial fusion machinery. *Mgm1* mutants are unable to grow in non-fermentable medium and display aberrant mitochondria with mtDNA loss and coalesced membranes.[14,15] Mutants lacking Msp1p show a phenotype similar to the *Mgm1* mutants. Msp1p was found to be anchored to the matrix side of the IMM and to be processed and regulated by proteases.[16] The mammalian homolog of Mgm1p is optic atrophy 1 (Opa1), which was found by screening for genes mutated in autosomal dominant optic atrophy (ADOA).[17,18] Disruption of Mgm1 or Fzo1 induces fragmentation and subsequent loss of mtDNA, both of which are antagonized by the loss of Dynamin related protein 1 (*Dnm1*), the first mitochondrial fission gene to be identified.

Dnm1p was discovered in a screening for genes within a collection of *Mdm* (mitochondrial distribution and morphology) mutants. The deletion of *mdm29-1*, similar to the mammalian dynamin 1 (*DNM1*) gene, caused mitochondrial elongation without mtDNA loss.[19] Given the role of dynamins in forming molecular collars that sever the endomembrane system, Dnm1p was hypothesized to be a mechanoenzyme involved in mitochondrial fission. Like the other mitochondria-shaping proteins, Dnm1p contains a GTPase domain that is required to maintain mitochondrial morphology.[19]

The ortholog of Dnm1p is the mammalian dynamin-related protein 1 (Drp1), the lack of which results in an interconnected mitochondrial network and aggregates caused by the impaired organelle severing.[20] Notably, Drp1 regulates the interaction of mitochondria with the endoplasmic reticulum (ER) and the morphology of the latter, a relationship demonstrated to be essential for fission.[21]

MECHANISMS DEFINING MITOCHONDRIAL SHAPE

Mitochondrial fission

The function of mitochondrial fission is to generate two or more daughter mitochondria, often characterized by different levels of membrane potential ($\Delta\psi_m$). Indeed, fission can sort mitochondria with low $\Delta\psi_m$ or irreversible mtDNA damage to autophagy.[22,23] This asymmetric apportioning sustains mitochondrial and cellular homeostasis. Fission is crucial for the distribution of old and young mitochondria required to maintain stemness[24]

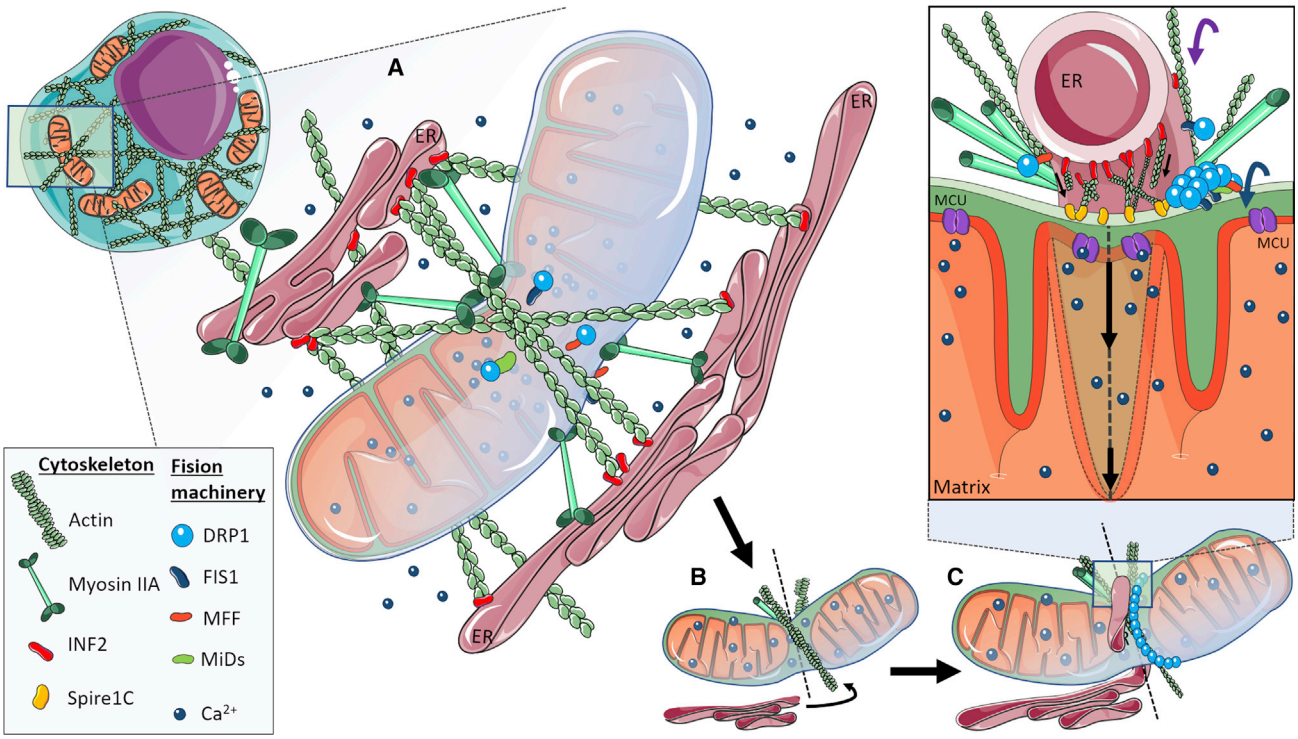

Figure 2. Mitochondrial fission coordinates with cytoskeleton rearrangements

(A) The cytoskeleton distributes actin and myosin to drive mitochondrial fission. In response to Ca^{2+} elevations, particularly those derived from the ER, actin polymerizes to form filaments throughout the cytosol. This is observed as different from the formation of actin clouds upon mitochondrial depolarization (see Figure 3) and is mediated by INF2.[51] INF2 sits at the ER, and upon activation by actin deacetylation, it helps distribute actin to sites of mitochondrial constriction generated by mechanical forces coordinated by an interstitial actin-myosin IIA network.[52]

(B) MCU (mitochondrial Ca^{2+} uniporter) allows mitochondrial Ca^{2+} elevations that occur after actin recruitment to constrict the inner membrane, a process that does not require Drp1 but an active electron transport chain to engage membrane partition (black arrows in the inset).[51]

(C) The ER elongates to wrap mitochondria at sites of fission (dotted line), as detailed in the inset: nucleation and polymerization of actin from INF2 at the ER membrane toward Spire1C in the mitochondrial outer membrane (short arrows).[41,44] The final step of fission depends on Drp1 recruitment by its interaction partners (FIS1, MFF, and MiDs) and its subsequent polymerization (blue arrow) that excises the mitochondrial outer membrane.

and for the even sorting of damaged mitochondria to daughter cells by actin waves during mitosis.[25] In addition, fission mediates the distribution of mtDNA to daughter mitochondria.[26–28] Indeed, replicating mtDNA is associated with areas of mitochondrial constriction and mitochondrial-ER contact sites (MERCs) that mark fission sites. However, selection of healthy versus mutated mtDNA for transmission to daughter mitochondria appears to be mostly influenced by mitochondrial cristae, which limit mtDNA diffusion within a continuous mitochondrial network rather than by the position of the replicating mtDNA molecules.[29]

Fission is mainly exerted in mammals by the GTPase dynamin-related protein Drp1, recruited to mitochondria downstream of cytoplasmic signaling cascades and at mitochondrial contact sites with peroxisomes, lysosomes, endosomes, lipid droplets, and vesicles from the *trans*-Golgi (see Tábara and Prudent[30] for a review). Drp1 is a cytoplasmic protein, ubiquitously expressed and found in the cytosol associated with the retrograde motor protein dynein-dynactin complex.[31] Drp1 ablation results in mitochondrial elongation due to unopposed fusion.[32] Dynamin 2 (DNM2) has been proposed as responsible for the final scission of mitochondria at sites marked by the ER.[33] However, its ablation does not inhibit fission[34–36] (see Kraus et al.[37] for a review). Thus, Drp1 acts as the master regulator of fission, both constricting tubular membranes and representing the minimum constituent sufficient for the severing of membranes.[35]

The ER marks and constricts the sites (~300–500 nm in diameter) that will accommodate ~120-nm-diameter rings of Drp1 to split mitochondria.[38–40] Primed by the wrapping of ER membrane extensions around mitochondria, actin interacts mainly at the ER surface with the ER-localized formin 2 (INF2). INF2 cross talks with the actin-nucleating protein Spire1C to promote actin assembly at the mitochondrial surface,[41] as well as with myosin II,[42–44] although the SNAP Receptor (SNARE) protein syntaxin 17 (SYN17) may also be involved[45] (Figures 2A–2C). Myosin IIA may provide the mechanical force that drives the initial mitochondrial constriction by actin cables[42,43] in the absence of OMM discontinuity[46–48] (Figures 2B and 2C). Moreover, myosin IIA and actin participate in the recruitment and oligomerization of Drp1 to mitochondria to trigger fission. Upon stimulation of fission, OMM-recruited actin oligomerizes to activate this already mitochondrially translocated Drp1.[49] Fission does not occur or can be reverted in the absence of actin polymerization,[49] which is required to exert membrane tension during fission.[50]

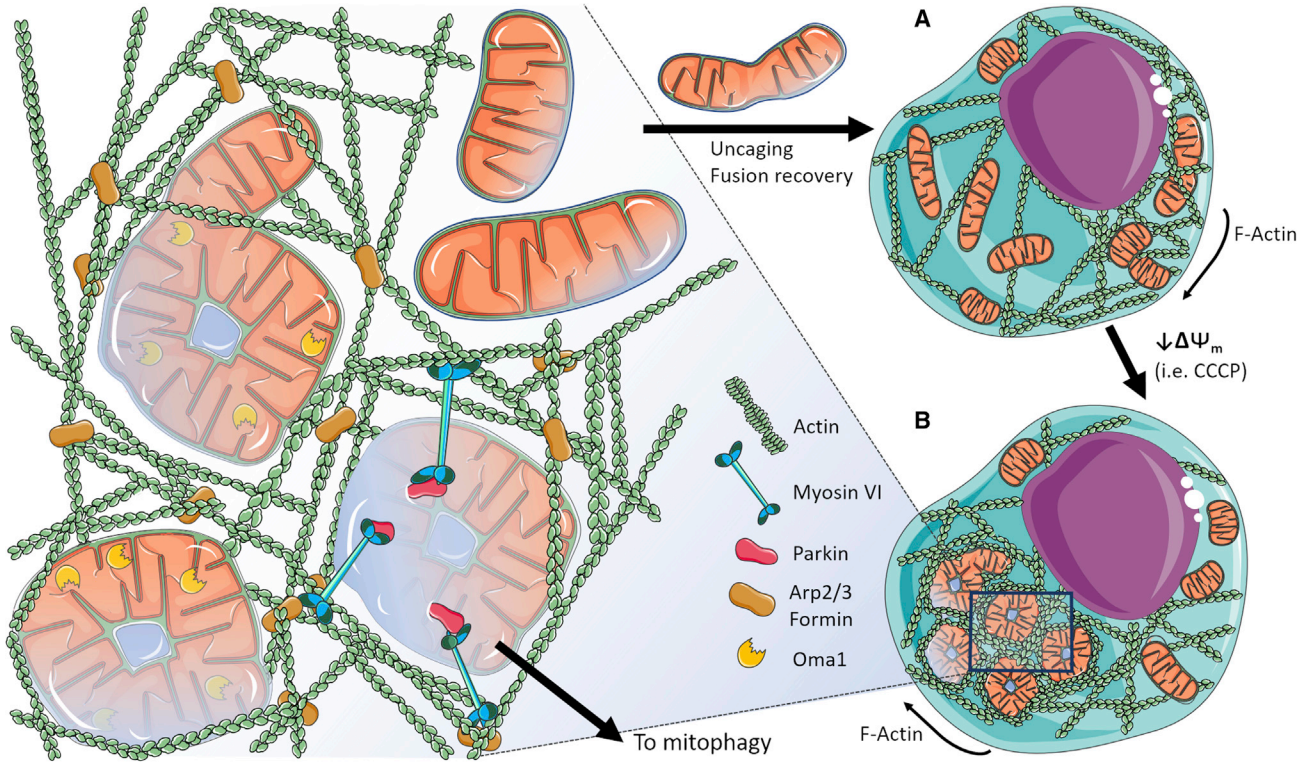

Figure 3. Actin cycling and trapping of depolarized mitochondria regulate fission and mitophagy

(A) The thin arrows show the cellular distribution of actin following a dynamic and cyclic association with subsets of mitochondria. This process requires the participation of Arp2/3 and formins and coordinates with Drp1 to foster fission at defined cellular topologies where actin is more abundant.[43]

(B) Upon depolarization, F-actin impinges in Arp2/3 to form a cloud around mitochondria that differs from INF2-mediated filamentation observed in response to mitochondrial Ca^{2+} overload (see Figure 2).[53] Depolarized mitochondria present a circularized matrix occurring with Oma1 activation (detailed view), independently of changes in the outer membrane or in the ability to recruit actin.[53] The actin network cages mitochondria in coordination with Arp2/3, formins, and myosin VI, the latter recruited by interaction with Parkin at the outer mitochondrial membrane (OMM) and leading to mitochondrial isolation before mitophagy occurs. In cases where mitochondrial depolarization is transient, mitochondria repolarize and are uncaged to undergo fusion and functional recovery.

To sever mitochondria, OMM and IMM fission must be coordinated. The initial IMM changes are linked to Ca^{2+} and INF2-induced actin polymerization at ER-mitochondrial contacts. The subsequent constriction by actin is maximized at the IMM by mitochondrial Ca^{2+} taken up from the ER. At the OMM, the constriction is engaged by the recruitment of Drp1, which is facilitated by the Ca^{2+}- and INF2-mediated assembly of actin filaments[42,51] (Figure 2C). Actin clouds are also formed in an Arp2/3 complex-dependent manner around depolarized mitochondria, but in this case, Drp1-driven fragmentation is not initiated. These depolarized mitochondria acquire a circular form mediated by the IMM protease Oma1[53] (Figure 3). Oma1 is a membrane depolarization-activated protease that cleaves and inactivates Opa1, as we will see in detail below. Thus, Oma1 can act as a rheostat that switches fusion off when fission is inactive and membrane potential is low. Indeed, in cells lacking Drp1, transient mitochondrial depolarizations activate Oma1 and lead to Opa1 cleavage, limiting the otherwise unopposed fusion.[54]

The importance of mitochondrial fission for development and adult life is substantiated by the severe phenotypes of Drp1 knockout mice. Because of defects affecting the neural tube, liver, and heart, Drp1 deletion is ultimately embryonically lethal.[55] Tissue-specific Drp1 ablation similarly causes severe phenotypes: when Drp1 is deleted in oocytes, female sterility ensues.[56] If Drp1 is ablated in the heart, mice display dilated cardiomyopathy, and if it is deleted in neurons, neurite outgrowth and synapse formation are affected.[2] Similarly, human inactivating *DRP1* mutations lead to extremely severe clinical outcomes, including lethal neonatal microcephaly, hypoplasia, optic atrophy, lactic acidosis, and elevated very long-chain fatty acids in serum.[32] Because Drp1 is also essential for peroxisomal shape and function, it is unclear how much these severe phenotypes arise from mitochondrial or peroxisomal defects.[32,57]

Drp1

Structure. Drp1 is characterized by an N-terminal GTPase domain, a dynamin-like middle domain, a variable domain, and a C-terminal GTPase effector domain (GED). The GTPase domain connects to bundle signaling elements (BSEs) and to a stalk domain responsible for Drp1 binding to membranes, oligomerization, and conformational changes that ultimately sever the mitochondrial membrane.[40,58] The central stalk in the middle domain of Drp1 drives the formation of multimers[58,59] around the fission sites marked by the ER, where Drp1 subsequently hydrolyzes GTP to constrict and sever mitochondria.[39,60]

Regulation. Several post-translational modifications regulate Drp1 recruitment to mitochondria and activity: phosphorylation, ubiquitylation, SUMOylation, O-GlcNAcylation, and nitrosylation.[38]

One of the most studied Drp1 regulatory mechanisms is phosphorylation, which occurs at multiple sites and can either promote or inhibit fission. In response to bursts in cytosolic cAMP, protein kinase A (PKA) reversibly phosphorylates Drp1 on S637 to block its GTPase activity and thus fission.[61,62] During autophagy, this pathway triggers mitochondrial elongation to spare mitochondria from degradation.[3] Phosphorylation of S637 by Pim-1 also occurs upon Drp1 O-GlcNAcylation in hyperglycemia and lowers Drp1 mitochondrial recruitment.[63] Conversely, phosphorylation by Ca^{2+}/calmodulin-dependent protein kinase Iα (CaMKIα) and Rho associated coiled-coil containing protein kinase 1 (ROCK1) during hyperglycemia or excess Ca^{2+} causes fission and cell death.[64,65] Drp1 S637 is dephosphorylated by the Ca^{2+}-dependent phosphatase calcineurin (CaN) in physiological conditions or by phosphoglycerate mutase family member 5 (PGAM5) during cell death to promote fission.[61,66,67] The same S637 residue can also be SUMOylated by the mitochondrial anchored SUMO E3-ligase mitochondrial E3 ubiquitin protein ligase 1 (MAPL). The SUMO1 protease SUMO specific peptidase 5 (SENP5) counteracts this modification, and its overexpression in cardiomyocytes results in hyperfused mitochondria, causing dilated cardiomyopathy *in vivo*.[68]

The other major site for Drp1 regulation is S616. During mitosis, it is phosphorylated by cyclin dependent kinase 1 (CDK1)/cyclin,[69] while during cell reprogramming or tumorigenesis, it is phosphorylated by mitogen-activated protein kinase 1 (MAPK1).[70] Pim1 antagonizes Drp1 phosphorylation at S616 by MAPK1.[71] Dual specificity phosphatase 6 (DUSP6), glycogen synthase kinase 3 beta (GSK3B), or protein kinase C (PKC) can also regulate Drp1 at other phosphorylation sites, as reviewed elsewhere.[72,73]

Drp1 levels can be regulated transcriptionally or by controlling its protein stability. Noncoding RNAs, like micro-RNAs (miRs), can indeed impinge on Drp1 transcription. The p53-controlled MiR-30 is downregulated during apoptosis to increase *Drp1* expression.[74] MiR can also control fission indirectly by regulating can, which activates Drp1. Mechanistically, during heart ischemia, the p53-dependent miR-499 represses CnA and ultimately fission, conferring cardioprotection.[66,75] Controlling Drp1 degradation is another way to regulate mitochondrial fission. Loss of the crucial mitophagy E3 ligase Parkin increases Drp1-dependent mitochondrial fission, possibly via an increase in Drp1 levels.[76] Membrane associated ring-CH-type finger 5 (MARCH5) can also ubiquitinate Drp1 to induce fission[77]; however, it is unclear if this E3 ligase operates directly at the level of Drp1 or if it affects its adaptors on the OMM.

Adaptors of Drp1. Drp1 requires OMM adaptor proteins to be recruited to fission sites. In mammals, these adaptors include fission 1 (Fis1), mitochondrial fission factor (Mff), and mitochondrial division (MiD) 49 and 51.[78–80]

Mff is the best characterized Drp1 adaptor. It recruits Drp1 to mitochondria for steady-state fission, facilitating it at mitochondrial midzones but not at their edges.[23] Mff knockdown compromises Drp1 recruitment and fission.[79,80] In response to high AMP levels caused by mitochondrial dysfunction, protein kinase AMP-activated (AMPK) phosphorylates Mff, contributing to its pro-fission effects.[81]

On the other hand, Fis1 was the first Drp1 adaptor to be described in mammalian cells, in analogy to the role of Fis1p as a Dnm1p adaptor in yeast. However, it appears that Fis1 in mammals recruits Drp1 only in certain cell types and under specific conditions.[59,79,82–86] Nevertheless, Fis1 overexpression in mammalian cells invariably results in mitochondrial fission. One potential explanation for this effect can be the ability of Fis1 to bind and inhibit the GTPase activity of the pro-fusion proteins Mfn1, Mfn2, and Opa1.[87] Alternatively, full-length Fis1 might cause fragmentation as a consequence of mitochondrial dysfunction and Ca^{2+} overload.[88] A unifying theory was recently put forward to explain the role of Fis1 and Mff in mitochondrial fission.[23] In this model, two different modes of fission exist: both require Drp1, occur at similar rates, and are apparently not influenced by MiD49 and MiD51. At the mitochondrial periphery, Fis1 and mitochondria-lysosome contacts orchestrate the excision of small, depolarized mitochondria with high levels of reactove oxygen species (ROS) and Ca^{2+}, which often lack replicating DNA and are degraded by mitophagy. At the mitochondrial midzone, fission mostly relies on ER and INF2-recruited actin as constriction machinery, severing mitochondria for biogenesis. Thus, Fis1 appears to be more of a recruiter of lysosomes for peripheral fission and an adaptor for mitophagy than a specific Drp1 receptor.[23,83,89] This concept is in agreement with the role of Fis1 in the regulation of mitochondria-lysosome contacts and fission by recruiting Tre-2-budding uninhibited by benzimidazole-cell division cycle 16 domain, domain family member 15 (Tbc1d15), which stimulates GTP hydrolysis by Rab7 to untether mitochondrial-lysosomal contacts.[90,91]

While Mff and Fis1 also participate in the recruitment of Drp1 to peroxisomes, MiD49 and MiD51 are mitochondria-specific Drp1 adaptors that trigger fission.[92] High levels of these adaptors may sequester Drp1 and even inhibit fission.[93,94] In general terms, Mff and MiDs could have complementary roles in mediating Drp1 recruitment and mitochondrial fission: MiDs can recruit Drp1 to facilitate its oligomerization, while Mff recruits oligomeric and active forms of Drp1.

Mitochondrial fusion

During mitochondrial fusion, two (or more) mitochondria get in close contact, thanks to their ability to move along microtubules, tether in *trans*, and fuse their OMM and then their IMM. Fusion of OMMs involves an irreversible GTPase-driven fusion reaction initially exerted via homo-oligomeric or hetero-oligomeric complexes of mitofusins (Mfn) 1 and 2.[95–97] The following fusion of the IMM is driven in mammals by Opa1, and it allows sharing of matrix material between the fused mitochondria, i.e., mtDNA, lipids, proteins, metabolites, and ions[98,99] (Figure 4). Complementation and sharing of transcripts can even rescue mitochondrial dysfunction derived from mtDNA mutations. However, it should be noted that the sharing of IMM content through cycles of fusion and fission is relatively less efficient than the diffusion of OMM components, suggesting that the IMM is a diffusion-restricted compartment. This might be a consequence of

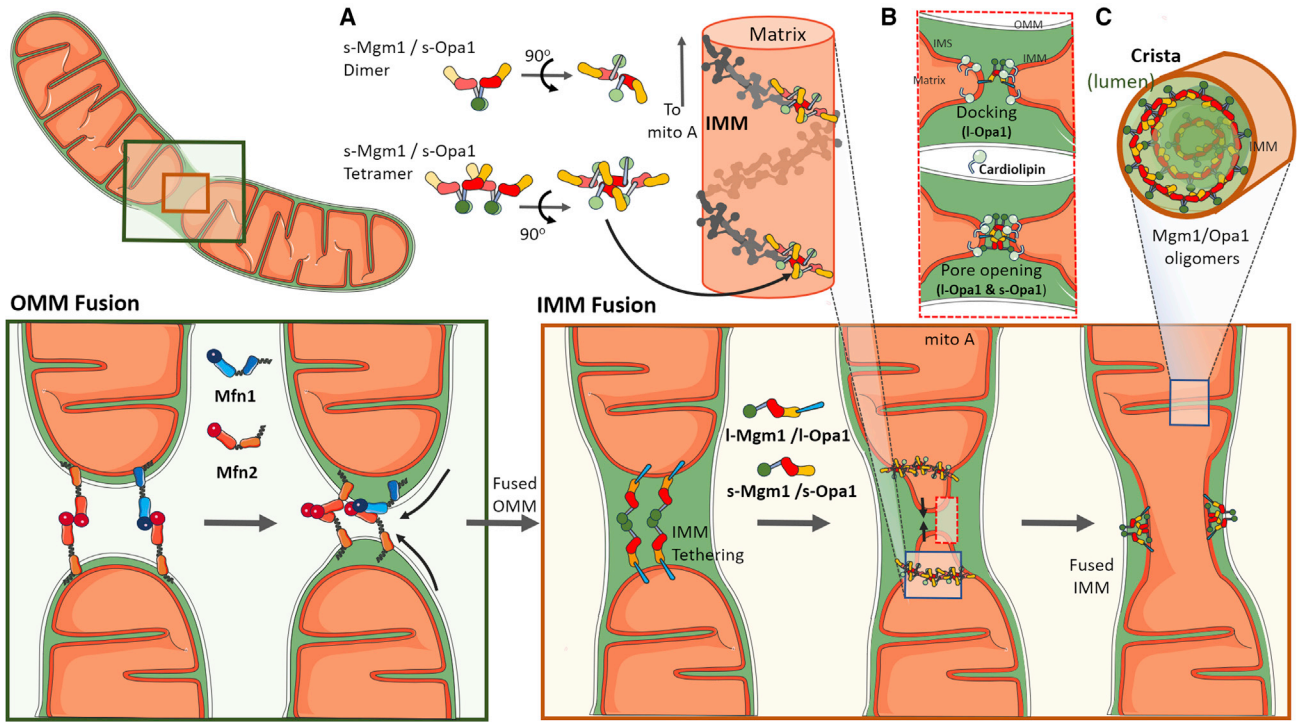

Figure 4. Mitochondrial outer and inner membrane fusion

(A and B) The sequential fusion of the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM) is shown from left to right. Note that both OMM and IMM fusion depend on the effective hydrolysis of GTP by the respective shaping proteins to account for the effective fusion of membranes. Homotypic or heterotypic Mfn1 and Mfn2 combinations tether and dock the OMM before its effective fusion. Fusion allows the sharing of components of the OMM and the intermembrane space (IMS) from the original mitochondria into a newly elongated one. Once the OMM is fused, long forms of Opa1 (l-Opa1) capitalize on an initial tethering of the IMM followed by a docking step. The interaction of l-Opa1 and cardiolipin at opposite sites is required for effective IMM fusion, which is fostered by heterotypic interactions between l-Opa1 and short Opa1 (s-Opa1) (B) and GTP hydrolysis.[101] These steps may be coordinated with the initial formation of oligomers at the IMM, as reported for Opa1[102] and its yeast ortholog Mgm1.[103,104] As shown in (A), s-Mgm1/Opa1 assemble into dimers as building blocks and subsequently tetramers that grow into helical filaments to form a lattice at the IMM, facing the intermembrane space. This occurs at buds in *trans* from the two fusing mitochondria and helps stabilize the curvature of the membrane at the fusion site. After fusion of the IMM (bottom right), l-Opa1 and s-Opa1 are sorted to both sides of the fused site.

(C) In the same scheme, an inset shows the distribution of s-Mgm1/Opa1 anchored in the luminal side of cristae to form helical structures that constrict (left-handed helixes) or expand (right-handed helixes) cristae to regulate their dynamic transitions and stabilize them in coordination with the constricting activity of MICOS and ATPase.[103,104]

the extreme cristae compartmentalization,[100] as discussed in detail below.

Like fission, mitochondrial fusion can be regulated by cytosolic signals.[105] For example, fusion is required to sustain ATP biosynthesis for the increased demands in the early steps of apoptosis, mitotic progression, or cellular senescence. The relevance of fusion for organismal health is further substantiated by the embryonic lethality in mouse models lacking Mfn1, Mfn2, and Opa1[106–108] and by the severe genetic diseases caused by mutations in *MFN2* and *OPA1* in humans.[9]

Opa1

The large GTPase Opa1 is the only dynamin-like protein discovered so far in the IMM. Opa1 is essential for mitochondrial elongation, resulting in fragmentation and mtDNA loss if absent.[109] *OPA1* mutations were identified as the most common cause of ADOA,[17,18] and *Opa1* loss of function in the mouse, which results in embryonic lethality.[106]

In humans, eight alternative splice variants of Opa1 exist. The mature Opa1 protein can be proteolytically cleaved at three sites by the metalloproteases YME1 Like 1 ATPase (YME1L1) and overlapping with the M-AAA protease 1 (OMA1). A non-canonical cleavage may also be exerted by presenilin associated rhomboid like (PARL), which originates a soluble IMS fraction that complexes with the IMM-anchored l-Opa1 to keep cristae junctions (CJs) tight (reviewed by Sprenger and Langer[110]).

Opa1 is required on only one IMM of the two opposing mitochondria to drive fusion[96] (Figures 4A and 4B). Indeed, Opa1 can bind in *trans* to the lipid cardiolipin (CL) in the IMM of the opposing mitochondria, as elegantly demonstrated by the group of Ishihara.[111,112] In this minimal model, Opa1 and CL heterotypically interact and require Opa1-mediated GTP hydrolysis to mediate fusion but not tethering.[113] However, this scenario is complicated in intact mitochondria, where Opa1 requires Mfn1 but not Mfn2, probably as a way to coordinate IMM and OMM fusion.[114] Indeed, Opa1 can also co-localize with Mfn1 at mitochondrial-ER contacts that mark points of fusion.[115] In yeast, the protein Ugo1p coordinates the fusion of both membranes by bridging Mgm1p and Fzo1, the homologs of Opa1 and Mitofusins (Mfns), respectively.[116,117] In mammals, Slc25a46 has been suggested as the ortholog of Ugo1p. Slc25a46 interacts

with Opa1, Mfn2, and the mitochondrial contact site and cristae organizing system (MICOS) component mitofilin (Mic60). SLC25A46 deficiency leads to altered cristae structure and lipid transfer from the ER,[118] potentially explaining its paradoxical pro-fission activity.[119]

Structure. Opa1 possesses an N-terminal domain comprising a mitochondrial targeting sequence (MTS) for mitochondrial import, a transmembrane domain that anchors it to the IMM, and a coiled-coil domain. The bulk of the protein is exposed to the IMS and comprises the GTPase domain, a middle domain, and a GTPase effector (GED) or assembly domain.[120]

As mentioned above, *Opa1* undergoes alternative splicing, producing in humans eight different transcripts that are all anchored to the IMM. These isoforms are called long Opa1 (l-Opa1), and they can be cleaved by the metalloproteases OMA1 and YME1L1 at the so-called S1 and S2 sites to generate five short forms of Opa1 (s-Opa1).[110,121]

Insights on Opa1 structure can be gathered, thanks to the availability of the structure of its yeast ortholog Mgm1p[103] (Figures 4A and 4B). In Mgm1p, four domains can be identified: a GTPase, a BSE, a stalk, and a paddle domain. A hydrophobic interface between opposite Mgm1 stalks is involved in the formation of V-shaped dimers, required to tubulate liposome membranes, to stimulate Mgm1 GTPase activity, and ultimately for its biological function.[109] Mgm1p dimers further assemble in tetramers characterized by a curvature radius compatible with the formation of helices assembled in a lattice. These helices can be left handed (in convex membranes, i.e., the cristae lumen) or right handed (in curved membranes like the inner boundary membrane). The gyration of the Mgm1p helices is therefore compatible with the two different biological functions of Mgm1p: when GTP hydrolysis occurs, the left-handed helices can constrict the cristae, whereas the right-handed ones can expand the IMM for fusion[103] (Figures 4B and 4C). In a structural study of mammalian s-Opa1, the same left-handed assembly of Mgm1p inside a lipid tube was observed for s-Opa1 decorating the outside of lipid tubes. This s-Opa1 was capable of tubulating liposomes irrespective of its GTPase activity, suggesting that s-Opa1 lattices display intrinsic membrane deformation properties. However, binding of a non-hydrolysable GTP analog to s-Opa1 resulted in the relaxation of the structure and hence in decreased curvature of the tube, positing a role for the GTPase reaction in membrane remodeling by s-Opa1.[102] This latter finding is in agreement with the cell biology studies, the clinical evidence, and the structural studies of yeast Mgm1 underlying the importance of GTPase activity for the multiple Opa1 functions that require membrane deformation.[103]

Regulation. A key facet of Opa1 regulation is the alternative splicing of >30 exons constituting the *OPA1* gene in humans to generate eight mRNAs.[17] These different isoforms appear to display different biological functions, e.g., in the maintenance of mitochondrial fusion and ultrastructure.[122]

A second key aspect of mammalian Opa1 regulation is its cleavage by the proteases Oma1 at S1 and Yme1l1 at the S2 site. It is worth noting that S1 is found in all isoforms, whereas S2 is present only in four isoforms. Thus, only Oma1 can cleave all l-Opa1 forms and convert them into their respective s-Opa1 forms.[123] The proteolytic cleavage of Opa1 is induced by different types of stress (mitochondrial dysfunction, oxidation) or increased oxidative phosphorylation (OXPHOS) activity that respectively activate Oma1 or Yme1l1 and induce mitochondrial fragmentation by shifting Opa1 toward its s-forms.[124] Although l-Opa1 in *trans* is sufficient to tether, dock, and partially fuse membranes via its ability to interact with CL, homotypic *trans* l-Opa1-l-Opa1 interactions increase the efficiency of the process.[112] On the other hand, s-Opa1 can only tether membranes. Importantly, the l-/s-Opa1 ratio is critical for fusion, as excess s-Opa1 blocks it, probably by outcompeting homotypic s-Opa1 tethering.[101] In the cellular context, l-Opa1 is sufficient to sustain fusion, even during stress.[124,125] The ablation of Yme1l1 results in fragmentation, but this phenotype results from Oma1 hyperactivation that leads to l-Opa1 cleavage and s-Opa1 accumulation.[124,126] Whether the accumulation of s-Opa1 triggers fission via the participation of s-Opa1 in IMM severing or by facilitating the assembly of the fission machinery at the OMM, or by impairing the fusion activity of Opa1 remains unclear. Indeed, s- and s-Opa1 forms must cooperate to increase the poor fusion efficiency of either form alone.[127]

Metabolic and bioenergetic signals can transcriptionally control Opa1. For example, the AKT serine/threonine kinase (Akt)-mechanistic target of rapamycin kinase (mTOR)-nuclear factor kappa B (NFκB) axis can be activated by insulin to recover respiratory capacity and bioenergetics along with increased Opa1-dependent mitochondrial elongation.[128] Indeed, NF-κB and its activators tumor necrosis factor α (TFNα) and inhibitor of nuclear factor kappa B kinase subunit β (IKKβ) upregulate Opa1 transcripts.[129] Conversely, mTOR complex 1 (mTORC1) activation protects from proteolytic inactivation of Opa1 in a process requiring Mfn2.[130] Opa1 is also subjected to O-GlcNAcetylation and ubiquitination.[35] Oxidative stress is also a direct regulator of Opa1 processing or oligomerization, the latter facilitated by the reactive oxygen species modulator (ROMO1) to preserve cristae stability and survival.[131]

Mfn1 and Mfn2

The OMM fusion factor Fzo1 was originally identified in *D. melanogaster* and yeast.[10,11] Two mammalian Mfns were then identified as Fzo1 orthologs.[132] The two Mfns are 80% identical and highly conserved, with the mouse proteins being 64% similar to the human ones.[97,132] Both proteins are ubiquitously expressed, maximally in the skeletal muscle and the heart.[1,105,133]

Mfns are abundant at contacts between adjacent mitochondria,[134] where they sequentially tether, dock, and increase the extension of OMM contacts by forming homotypic or more fusion-efficient heterotypic complexes (Figure 4). Because of this ability to form heterotypic complexes, either protein can rescue the genetic ablation of the other.[108,135] Moreover, the overexpression of either protein results in hypertubulation, i.e., extensive mitochondrial fusion and elongation, and in perinuclear mitochondrial aggregation.[134] Conversely, Mfns genetic deletion results in embryonic lethality, but the phenotypes of cellular models of individual Mfn ablation are not identical: mitochondria appear as short rods in *Mfn2*$^{-/-}$ cells, whereas they are spherical in *Mfn1*$^{-/-}$ cells.[108] The different phenotype may be explained by the higher efficiency of oligomeric Mfn1 to dock membranes and tether mitochondria, given that its GTPase activity is higher than that of Mfn2.[95,134]

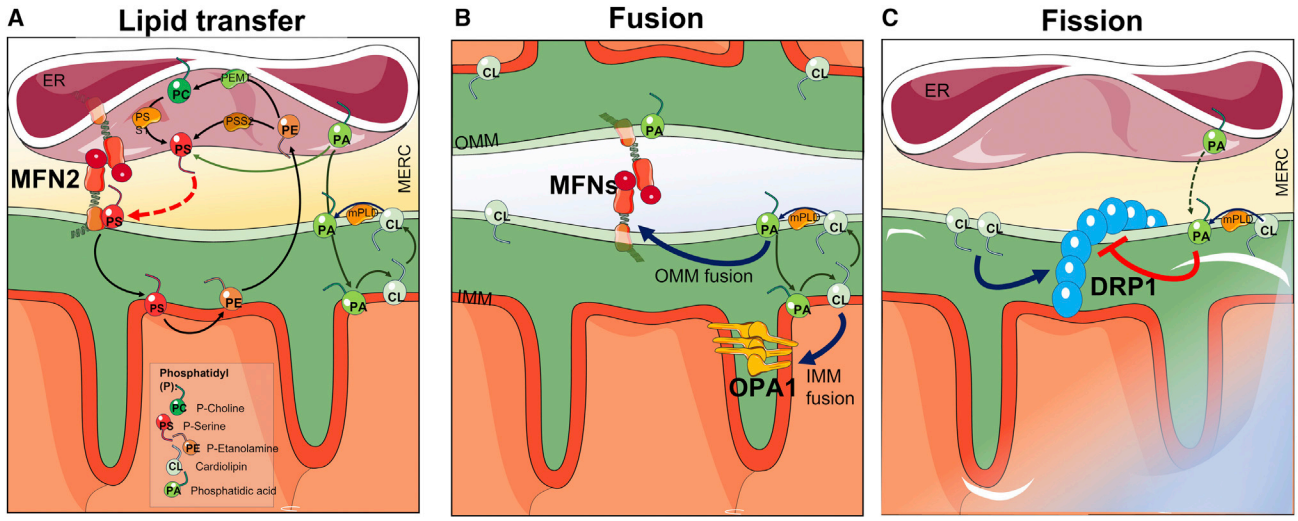

Figure 5. The role of phospholipid transfer at MERCs in the coordination of mitochondrial fusion and fission

(A) Phospholipid synthesis is topologically split between ER and mitochondria. In the ER, phosphatidylserine synthase 1 (PSS1) and 2 (PSS2) and phosphatidylethanolamine N-methyltransferase (PEMT) catalyze different steps converging at the synthesis of phosphatidylserine (PS). PS binds to and requires Mfn2 for its transfer to mitochondria and subsequent conversion to phosphatidylethanolamine, which is then transferred back to the ER.[140] Phosphatidic acid (PA), another lipid precursor synthesized at the ER, is sent to mitochondria to originate cardiolipin (CL) at the IMM. Although enriched at the IMM, CL can be relocated to the OMM and converted back to PA by the mitochondrial-localized phospholipase D (mitoPLD). PA and CL can respectively stimulate OMM and IMM fusion.
(B) Cardiolipin promotes mitochondrial fusion, particularly by acting as anchor for L-Opa1 in trans to fuse the IMM, which is further favored by S-Opa1 (see also Figure 4). The minimal machinery required for fusion to occur operates with CL in one mitochondrion and L-Opa1 in *trans*.[112] CL can be converted to PA by mitochondrial phospholipase D (MitoPLD), both acting to foster OMM fusion mediated by Mfns.
(C) In fission, CL stimulates Drp1 oligomerization and GTPase activity, acting together with Mff to facilitate fission. Reciprocally, Drp1 can promote CL clustering to favor division. Drp1 also binds to PA located at the OMM and probably also coming from the ER (dotted lines). PA blocks oligomerized Drp1 to impair fission.[141] The interaction of Drp1 with MitoPLD would provide a hub for the coordination of fusion and fission machinery by phospholipids (see Kameoka et al.[142] for a review).

In addition, Mfn2 has a crucial role in mitochondrial tethering with the ER, the sarcoplasmic reticulum, and other ER-derived organelles such as melanosomes.[136–139] ER-mitochondrial tethering by Mfn2 is particularly important for Ca^{2+} transfer from ER to mitochondria.[136,138,140] Importantly, Mfn2-mediated tethering facilitates phosphatidylserine (PS) transfer from the ER to the mitochondria, where it can be converted to phosphatidylethanolamine (PE).[140] This transfer between the ER and mitochondria is not only necessary for the synthesis and traffic of several phospholipids (Figure 5) but also determines fusion and fission, with CL and phosphatidic acid (PA) stimulating inner and outer membrane fusion, coordinated with respective stimulatory and inhibitory impact by CL and PA on Drp1-mediated fission (see Figure 5).[37]

Structure. Structurally, Mfns are characterized by an N-terminal part harboring the GTPase domain and a first coiled-coil domain (HR1), followed by a second coiled-coil domain (HR2) at the C-terminal part of the protein. A transmembrane region between the two HR accounts for OMM insertion.[97,132] The crystal structure of a dimerized minimal Mfn1 (comprised by the GTPase domain linked to the HR2) and a revised Mfn topology have revealed that the apposition and dimerization of N-terminal GTPase domains in *trans*, followed by GTP hydrolysis, are primary events for tethering and subsequent fusion of membranes.[143,144] It is noteworthy that the HR1 amphipathic helix is sufficient to insert and bind membranes in *trans* to drive bilayer fusion.[145] Supporting the above model, a peptide mimicking the HR1 helix is also able to induce fusion.[133] Such a model, however, is based on experiments performed with incomplete forms of the recombinant protein in solution that lack HR2.[113]

Dorn has recently proposed a unifying model. A first step of tethering operated by the C-termini of Mfns on mitochondria in *trans* would be followed by a *cis*-oligomerization of Mfn and a bridge in *trans* to an opposite toroid in the OMM of the partner mitochondrion.[146] In this scenario, the "closed" tethering unfavorable Mfn conformation accounts for the binding in *cis*, whereas the "open" tethering promoting conformation would be responsible for the *trans* binding of GTPase domains. The two toroidal rings that are formed would then be able to bend the OMM within the central hole and induce fusion by relaxing the induced membrane deformation. Finally, GTP hydrolysis would dissolve the toroids.[1]

Regulation. Several transcriptional, post-transcriptional, and post-translational regulators of Mfns define their ability to trigger fusion.

The peroxisome proliferator-activated receptor gamma coactivator 1-alpha (Pgc-1α), key for mitochondrial biogenesis, as well as estrogen-related receptor-alpha (Err-α) and Pgc-1β promote *Mfn2* expression.[147] During neuronal excitotoxicity, the transcription factor Mef2 is degraded, resulting in reduced *Mfn2* expression.[148] A handful of miRNAs (MiR-214, MiR-106b, Mir-761, and MiR-140) have been identified to modulate mitochondrial shape by downregulating *Mfn2*[149–151] or *Mfn1* expression.[152] These miRNAs provide means to epigenetically regulate mitochondrial fusion in several pathophysiological conditions and can explain the different mitochondrial morphologies observed, e.g., during cell cycle.

Post-translational modifications of Mfns include oxidation, ubiquitylation, and phosphorylation. Mitofusins can be regulated by the redox status of residues placed within the mitochondrial intermembrane space. Their oxidation can lead to Mfn oligomerization and subsequent fusion.[153] The ubiquitination of acetylated Mfn1 by MARCH5 targets the protein for proteasomal disposal during stress.[154] Conversely, the histone deacetylase 6 (HDAC6) enhances Mfn1-mediated fusion in response to glucose deprivation and oxidative damage adaptation.[155] MARCH5also ubiquitinates mitochondrial-bound Mfn2, but not ER-bound Mfn2, to facilitate Mfn2 oligomerization and mitochondrial-ER tethering.[156] Parkinson protein 2, E3 ubiquitin protein ligase (PARKIN) is another ubiquitin ligase that also tags Mfn1 for ubiquitination during apoptosis.[157] Its action can be reversed by the deubiquitylase USP30, thus recovering the mitochondrial fusogenic capacity.[158] Phosphorylation of Mfns can favor or prevent fusion depending on the phosphorylated site and the responsible kinase. The HR1 domain of Mfn1 can be phosphorylated by ERK to prevent fusion and facilitate Mfn1 binding to the Bcl-2 family member Bak, in response to intrinsic stimuli of apoptosis.[159] Jnk can phosphorylate Mfn2 under stress conditions as a required step for Huwe1 recruitment to activate the proteosomal degradation of Mfn2 and apoptosis.[160] All in all, these different layers of regulation highlight the importance of mitochondrial fusion-fission balance in cell physiology.

MECHANISMS DEFINING MITOCHONDRIAL ULTRASTRUCTURE

A key aspect of mitochondrial morphological organization is the intricate ultrastructure of the organelle. The presence of two membranes as well as the fact that the IMM is folded into cristae represents perhaps the most intricate organization of a biological membrane known so far, with profound implications in mitochondrial physiology, largely influenced by this ultrastructural organization.

The shape-function relationship at the mitochondrial ultrastructural level

A key feature of the cristae organization is the presence of CJs, the tubular membrane fraction that connects the bag-like body of the cristae to the inner boundary membrane. Importantly, CJs help retain the bulk of cytochrome *c* inside the cristae lumen.[161] The existence of CJs has a 2-fold effect on mitochondrial pathophysiology. First, by sequestering cytochrome *c* inside the cristae, CJs sustain localized respiratory chain function and generation of the electrochemical gradient.[162,163] Indeed, mitochondrial cristae accommodate the respiratory complexes in charge of pumping protons toward the intermembrane space to generate an electrochemical gradient used for ATP production.[163] Of note, individual cristae within a same mitochondrion behave as single units with heterogeneous membrane potential.[100] Second, CJs regulate apoptosis by impeding the egress of the bulk of mitochondrial cytochrome *c* that is required in the cytosol for apoptosis induction.[161,164] In view of these crucial functions, it is not surprising that cristae morphology and CJ stability are tightly controlled.[163,165,166] It shall be noted that cristae are not separate compartments from the inner boundary membrane, as partial and transient diffusion of membrane components between the inner boundary membrane and cristae occurs.[167]

A further key aspect of cristae organization is their dynamic nature. Such dynamicity allows us to respond to metabolic and bioenergetic demands by controlling the formation and the stability of respiratory supercomplexes (RSCs) and ATP synthase oligomers.[4,6,168] Pioneering work by Hackenbrock[169] described the reversible transition from an orthodox status with more compact cristae to a condensed status (i.e., increased intracristal space and an electrodense matrix with reduced volume), where respiration is activated for ATP biosynthesis. A second key finding was that cristae rearrangements also occur during apoptosis. While increased bioenergetic demands result in cristae tightening, apoptotic stimuli drive the widening of cristae to maximize the release of the nearly 80% of cytochrome *c* confined in the intracristal space and efficiently induce apoptosis.[161] Experiments with mutants of the pro-apoptotic BCL-2 family member Bid indicate that both cristae remodeling and mitochondrial membrane permeabilization (MOMP) are required for efficient apoptosis.[6] In keeping with this, cristae remodeling does not induce cell death in the absence of MOMP, instead, it causes bioenergetic changes due to the destabilization of mitochondrial RSCs and ATP synthase oligomers.[168,6] This discovery reinforces the essential role of mitochondrial cristae in the regulation of mitochondrial pathophysiology. Furthermore, it supports the intimate connection between controllers of apoptosis and regulation of mitochondrial ultrastructure, with far-reaching implications for human health.[170] Super-resolution microscopy detailed the ability of cristae to undergo fusion and fission events, leading to the suggestion that CJs can merge and split.[167] This study, together with several others,[100,171] opens novel avenues to address the relationship between mitochondrial ultrastructure dynamics and mitochondrial function. For example, mtDNA molecules concentrate in foci localized in voids between the lamellar cristae,[171] constituting a physical barrier for fission.

MICOS

The MICOS, which connects the IMM and OMM, is a crucial determinant of mitochondrial ultrastructure.

MICOS is composed of Mic10, Mic12, Mic19 Mic25, Mic26, Mic27, and Mic60 (Mitofilin/Fcj1). The silencing of any of these components results in a similar phenotype of CJ loss and the formation of onion-like cristae.[166,172,173] Gene deletion and biochemical studies have identified two subcomplexes: one formed by Mic10, 13, 26, and 27, and a second one by Mic60, 19, and 25.[166]

Mic60 is the central subunit of MICOS. It acts independently of OXPHOS and CL to scaffold CJs of the nascent cristae.[166,174] Mic60 is sufficient to fold cristae independently of OMM dynamics[175] and to induce IMM branching. Its genetic ablation results in onion-like mitochondria that lack the vast majority of CJs.[176] Mic60 establishes contacts between the OMM and IMM[176] and bends membranes, a shared feature with Mic10[166,176] that coordinates Mic60 distribution and oligomerization.[175]

Mic19 appears as an essential scaffold molecule: it can bridge Mic60 and Opa1 interactions in CJ formation,[177,178] and it interacts with the mitofilin domain of Mic60 to fit the convex CJ

membrane.[179] The Mic60-Mic19 tetramer may counteract excessive cristae curvature and CJ scission caused by the unopposed function of other scaffolds such as Mic10.[179] In the formation of contact sites between the IMM and the OMM, Mic19 bridges Mic60 with the protein import machinery component Sam50.[165,177] The interaction between MICOS and OMM components allows the coordination of cristae biogenesis with intracellular signaling and mitochondrial dynamics. Indeed, MICOS relies on Sam50 to interact with the actin motor Myo-19[180] and the mitochondrial molecular motors mitochondrial rho GTPase (Miro) 1 and 2.[179,181] Interactions with microtubules mediated by Miro1/2 prime the pulling of membranes to generate steady-state TOM20$^+$ mitochondrial-derived vesicles (MDVs).[182]

Opa1

Opa1 is a master regulator of cristae shape, independently from its effect on mitochondrial fusion.[164] *Opa1* gene ablation results in the loss of cristae and in widening of the lumen of the residual ones.[109,164,6,183] Opa1 regulates CJs to define in turn the cristae lumen width,[184] which is reduced when the protein is mildly overexpressed.[6,185] Opa1 does not operate alone in defining cristae shape. First, Opa1 and MICOS components physically and genetically interact in the same pathway, with some overlapping functions in the stabilization of the negative CJ curvature. Both proteins stabilize the cristae and prevent their detachment from the inner boundary membrane to generate isolated cisternae.[166,176] Mic60 and Opa1 are retrieved from the same complexes.[184] While MICOS is required to build cristae[166] and genetically interacts with Opa1, Opa1 acts upstream of Mic60 in defining the number and width of CJs.[184]

Opa1-dependent regulation of cristae shape impacts mitochondrial function. Genetic models of Opa1 ablation and mild overexpression, along with apoptotic cristae remodeling, shed light on how cristae morphology coordinates the assembly and stability of RCSs[6] and ATP synthase oligomers.[168,4] Notably, this occurs without variations in mtDNA copy number or a direct effect on ATP synthase, with which Opa1 can interact.[168,4] Increased Opa1 levels not only tighten cristae but also increase the growth of cells relying on mitochondria for ATP production,[6] such as during starvation[4] or stem cell differentiation.[186] Conversely, when respiration is compromised, cristae tightening by Opa1 may be beneficial to stabilize ATP synthase oligomers, thus facilitating the reversal activity of the ATP synthase to safeguard the mitochondrial electrochemical gradient[168] and prevent ROS accumulation and cell death.[187] Altogether, these data provide a mechanistic explanation for how regulation of cristae morphology by Opa1 counteracts secondary as well as primary mitochondrial defects *in vivo*.[185,188]

ATP synthase

The F_oF_1-ATP synthase (ATP synthase) is the only protein complex so far reported as sufficient to induce positive curvature (i.e., resembling a spheroid) at the apex of IMM invaginations, thus contributing to the origin of cristae.[189–191] ATP synthase dimers can "pinch" the IMM and subsequently expand it to form rows, disposed at the edges of planar or tubular cristae.[189,192] ATP synthase dimerization is also stimulated in conditions where higher mitochondrial ATP production is required: during aging,[193] starvation,[3] or limited glycolysis.[168,4,194]

ATP synthase dimers are essential to both stabilize the enzyme and induce the IMM curvature toward the matrix.[189,195] The genetic ablation of ATP synthase dimerization subunits (sub) *e*, *g*, and *k* results in the loss of cristae or in the generation of aberrant *septae* and onion-like structures.[165,168,190,191,196] Other ATP synthase subunits[189,191,197,198] and modulators of ATP synthase activity such as IF_1 may also interfere with dimerization and cristae structure.[199] These results indicate the degree of intertwining between the role of the ATP synthase in ultrastructure and in bioenergetics.

The absence of Opa1[168,4] or key MICOS components[173] results in the loss of ATPase dimers and formation of aberrant cristae,[168,190] supporting the idea that cristae originate from at least a tripartite regulatory network comprised by Opa1, MICOS, and ATP synthase. Indeed, several subunits of the ATP synthase interact with Opa1,[4] although the reduction of cristae lumen width by Opa1 is the key feature controlling the stabilization of RCSs and ATP synthase oligomerization.[168] ATP synthase can also physically interact with the MICOS subunit Mic10 but not Mic60.[173] Whether this interaction is required at the initial or at the final steps of cristae folding is still unclear.

Cardiolipin and cristae morphology

Lipid composition is per se a major determinant of membrane bending and of the stabilization of protein complexes, hence impacting cristae morphology.[200] A key lipid that characterizes the IMM and influences its shape is CL. The lack of CL, which is critical for the assembly of high molecular weight mitochondrial respiratory complexes, causes ultrastructural defects and loss of RCSs, as observed, for example, in cells from patients with Barth syndrome.[201] As mentioned above, CL can also participate in mitochondrial fusion, as the interaction between l-Opa1 and CL is sufficient to mediate it.[111,112] It is unclear, however, whether this lipid-protein interaction also directly participates in the stabilization of cristae morphology. So far, we have understood that CL can also influence Opa1 by impinging on its protease Oma1. In neurons, the CL-binding protein prohibitin 2 stabilizes CL, facilitating Oma1-CL binding and turnover. The turnover of Oma1 ultimately prevents Opa1 cleavage, cristae remodeling, and cytochrome *c* release.[202]

THE MITOCHONDRIAL OUTCOMES OF DYNAMICS

The relationship between mitochondrial shape and function

Following the path of pioneering studies performed in the 1960s,[169] increasing evidence supports the existence of an intimate relationship between mitochondrial shape and metabolism, culminating in the demonstration that cristae shape influences assembly and stability of RCSs to streamline respiration efficiency for cellular growth.[6]

Respiration

The concept that mitochondrial dynamics can impact respiration stems from the discovery that individual mitochondria within the same cell display heterogenous $\Delta\Psi_m$ and bioenergetic parameters. When fusion is promoted, $\Delta\Psi_m$ is more homogenously

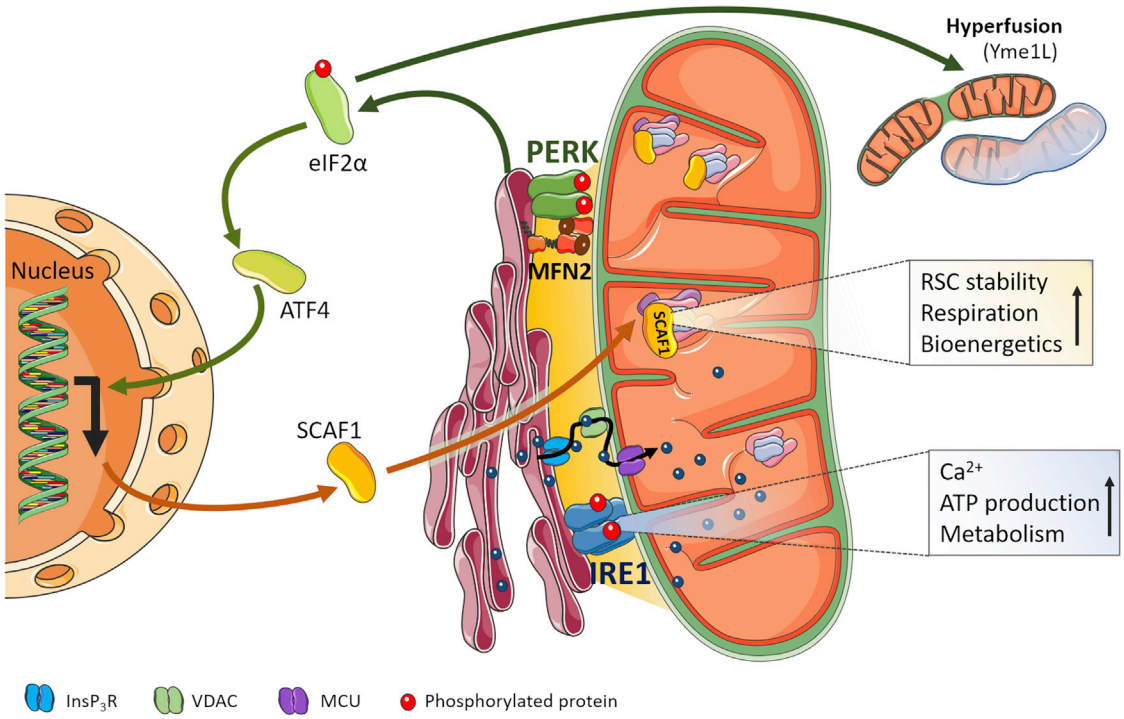

Figure 6. ER stress players in ER-mitochondria functional interactions

ER stress key players PERK and endoplasmic reticulum to nucleus signaling 1 (IRE1) coordinate a response that translates into mitochondrial functional outcomes. Under ER stress or glucose deprivation, phosphorylated PERK engages the activation of eIF2α and ATF4 to transcribe the assembly factor SCAF1, which increases the formation of respiratory supercomplexes and respiration. In turn, stabilized supercomplexes help meet the high request for ATP production and bioenergetic supply from the mitochondria in the absence of glycolysis.[206] PERK is located at and stabilizes mitochondria-ER contact sites (MERCs).[210,211] At MERCs, Mfn2 interacts with and acts upstream of PERK to suppress its activation under ER stress, with consequences in ROS production along with mitochondrial respiration, Ca^{2+} overload, and morphology.[210] Mfn2 also regulates the ATF6 and IRE1 branches of ER stress. IRE1 also represents a major regulator of MERC stability, where it interacts with the inositol triphosphate receptor ($InsP_3R$) to control its location and abundance at MERCs. This interaction facilitates mitochondrial Ca^{2+} elevations to engage metabolic adaptations and ATP production by mitochondria, which can be separated from its role in the unfolded protein response[212] (see also Quintana-Cabrera and Soriano[207]).

distributed, but differences among individual mitochondria are not eliminated.[5] The $\Delta\Psi_m$ heterogeneity may in turn activate fission at specific sites of the mitochondrial network, where the membrane potential is lower.[203,23] It is tempting to speculate that this regioselective fission occurs downstream of the generation of perimitochondrial microdomains of AMP by the depolarized portion of the mitochondrial network. These AMP microdomains would in turn activate local AMPK to phosphorylate and activate the Drp1 receptor Mff at sites of mitochondrial depolarization.[81] In addition, defects in the electron transport chain (ETC) lead to Opa1 cleavage and mitochondrial fragmentation. Thus, mitochondrial functional heterogeneity might also stem from impaired fusion that compromises mtDNA levels, synthesis of respiratory chain complexes, and ultimately respiration.[204]

The concept of ''regional'' mitochondrial bioenergetics extends to individual cristae as independent bioenergetic units within the same mitochondrion. Individual cristae can indeed undergo depolarization-repolarization cycles without affecting neighboring ones.[100] Molecularly, Opa1 cleavage does indeed facilitate the transition from hetero- to equi-potential cristae within the same mitochondrion.[100] MICOS components can similarly respond to changes in mitochondrial respiration. Mic60 senses OXPHOS changes, linking bioenergetic demands to cristae shape. When oligomerized, Mic10 interacts with MICOS, while unassembled Mic10 molecules directly interact with the subunits *e* and *k* to stabilize ATP synthase oligomers and adapt respiration and growth to metabolic demands.[205] Conversely, when Mic60 is overexpressed, it interacts with the ATP synthase subunits *e*, *k*, and *g*, destabilizing oligomers and originating branched cristae and widened CJs.[166,176]

From a morphological standpoint, elongated mitochondria are usually linked to increased respiration and ATP production. This elongation occurs because of reduced fission or increased fusion.[3,4,5] Irrespective of the molecular mechanism, elongation is usually triggered by limited nutrient availability, further strengthening the concept that the cell adapts an organellar shape to respiratory needs. Of note, mitochondria-ER communication can regulate the stability of RSCs by engaging the transcription of the RSC assembly factor SCAF1 through the ER stress axis proline-rich receptor-like protein kinase 1 (PERK1)-eukaryotic translation initiation factor 2A (eIF2α)-activating transcription factor 4 (ATF4).[206,207] PERK-eIF2α can induce Yme1l1-mediated mitochondrial hyperfusion to accommodate more RSCs[208] (Figure 6). PERK also contributes to cristae organization by facilitating MIC19 entry into mitochondria.[209]

Mitochondria-shaping proteins, also of the OMM, can participate in the regulation of mitochondrial bioenergetics. While Mfn1 deletion seems to marginally affect it,[213] Mfn2 plays a more important role. In cells lacking Mfn2, proton leak increases, coenzyme Q is lost, $\Delta\Psi_m$ is lowered, and ROSs are produced.[73,214–217] The differences between Mfns might be explained considering the role of Mfn2 in ER-mitochondria tethering, impacting interorganellar transfers of lipid and calcium that stimulate the Krebs' cycle dehydrogenases and hence mitochondrial respiration.[136,138,140,218]

Ion exchange across the IMM

Given that mitochondrial dynamics impacts proton-motive force (Δp) generation and maintenance, it is not surprising that it also affects ion fluxes across the IMM. Because these fluxes also influence Δp, some of the reported effects of mitochondrial dynamics on the electrochemical gradient (more commonly measured as effects on $\Delta\Psi_m$) can reciprocally be explained as epiphenomena of dynamics-induced changes in ion fluxes.

Perhaps the largest effect on the morphology of the organelle is observed upon changes in K^+ fluxes across the IMM. Indeed, changes in K^+ homeostasis lead to mitochondrial fragmentation,[219] and mitochondria lacking the mitochondrial K^+_{ATP} channel are massively fragmented and almost completely devoid of cristae.[220] In this context, leucine zipper and EF-hand containing transmembrane protein 1 (Letm1), which has been proposed as a crucial component of the K^+/H^+ antiporter,[221] has been consistently reported as an Opa1 interactor in unbiased proteomics catalogs (for example, Piao et al.[222]). Whether the interaction between Letm1 and Opa1 is responsible for the coordination of K^+ fluxes with changes in ultrastructure and morphology of the organelle is a fascinating question that remains to be addressed. Reciprocally, whether fusion of the IMM orchestrated by Opa1 leads to changes in its permeability awaits understanding from the bioenergetic, electrophysiological, and molecular perspectives. For example, pH flashes that propagate among adjacent mitochondria to equilibrate their membrane potential require Opa1 and occur much faster than mixing of matricial content,[223] suggesting that the events of mitochondrial IMM fusion are coupled to fusion pores of different sizes with different diffusion filters. This hypothesis is however challenged by the finding that these pH flashes persist in mitochondria expressing only the fusion-incompetent $Opa1^{K301A}$ mutant,[164,224] calling for further studies on how Opa1 regulates matricial pH changes.

CONCLUSIONS AND PERSPECTIVES

In the last 20 years, we have witnessed the birth and expansion of the field of mitochondrial dynamics. However, this area of research is far from being mature. A key question that has not been tackled so far is how mitochondria can fuse their inner membrane without dissipating the membrane potential. This is linked to the other question of whether a fusion pore is established during fusion of the IMM. We believe that the integration of *in vitro* systems and patch-clamp approaches might shed new light on this process. Similarly, it is unclear if mitochondrial fission can start from the IMM. Are there yet unidentified players that can constrict mitochondria from the interior? If yes, how is their function coordinated with the canonical fission machinery? Campaigns to identify other molecules regulating mitochondrial morphology and ultrastructure in an unbiased fashion are also needed. We think that high content screenings using potent readouts can expand a core machinery that so far remains limited to a very small set of proteins. For example, unbiased screening shows that lowering mitochondrial CL levels can counteract the mitochondrial fission observed in cells with mutated, inactive Opa1.[225]

The role of mitochondrial shape in tissue function and dysfunction is largely studied using gene deletion approaches. However, as we stated above, the field is ripe for more refined approaches where changes in mitochondrial shape during a given pathophysiological process are specifically blunted, e.g., by interfering with the upregulation or downregulation of the mitochondria-shaping protein involved. Such a specific approach would clarify if changes in levels, more than the presence, of a specific mitochondria-shaping protein are required in pathophysiology.

Finally, we expect that the field will branch out to understand the function of mitochondrial morphology in very complex disorders, including cancer, and that therapeutic tools will be identified and utilized in experimental therapeutics for a variety of conditions.

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DECLARATION OF INTERESTS

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