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Fusion and Fission: Interlinked Processes Critical for Mitochondrial Health

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Abstract

Mitochondria are dynamic organelles that continually undergo fusion and fission. These opposing processes work in concert to maintain the shape, size, and number of mitochondria and their physiological function. Some of the major molecules mediating mitochondrial fusion and fission in mammals have been discovered, but the underlying molecular mechanisms are only partially unraveled. In particular, the cast of characters involved in mitochondrial fission needs to be clarified. By enabling content mixing between mitochondria, fusion and fission serve to maintain a homogeneous and healthy mitochondrial population. Mitochondrial dynamics has been linked to multiple mitochondrial functions, including mitochondrial DNA stability, respiratory capacity, apoptosis, response to cellular stress, and mitophagy. Because of these important functions, mitochondrial fusion and fission are essential in mammals, and even mild defects in mitochondrial dynamics are associated with disease. A better understanding of these processes likely will ultimately lead to improvements in human health.

OVERVIEW OF MITOCHONDRIAL BEHAVIOR

Mitochondrial DNA (mtDNA): the circular mitochondrial genome that encodes 37 genes, comprising 13 polypeptides of the respiratory chain, 2 ribosomal RNAs, and 22 transfer RNAs

GTPase: an enzyme that binds to and hydrolyzes guanosine triphosphate (GTP)

The complex and dynamic behavior of mitochondria in living cells has been known for almost a century. In 1914, a careful analysis of live chick embryonic cells revealed that mitochondrial "granules can be seen to fuse together into rods or chains, and these elongate into threads, which in turn anastomose with each other and may unite into a complicated network, which in turn may again break down into threads, rods, loops and rings" (73, p. 332). These elegant, early descriptions of mitochondrial fusion and fission portrayed organelles that engaged in intricate but mysterious behavior of unknown function. The molecular basis of these behaviors remained unknown until the late 1990s, when the first molecules mediating mitochondrial fusion and fission were discovered.

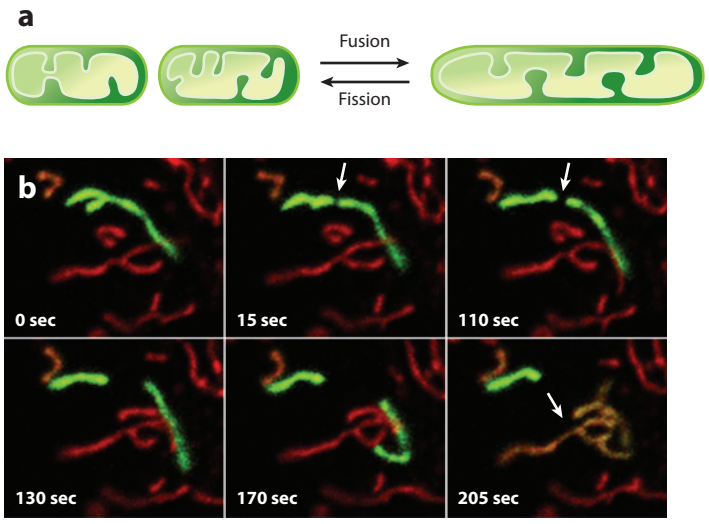

Figure 1

Mitochondrial fusion and fission. (*a*) A schematic of mitochondrial fusion and fission. During mitochondrial fusion, the two outer membranes and the two inner membranes must coordinately merge. (*b*) Live imaging of fission and fusion events. Mouse embryonic fibroblasts were transduced with retrovirus expressing matrix-localized mito-DsRed and mito-photoactivatable green fluorescent protein (mito-PA-GFP). Illumination of a small region with a 405-nm laser results in bright mito-PA-GFP fluorescence (*green*). Between the first and second frames, the green mitochondrion undergoes fission, and the daughters move apart. In the last frame, one of the daughter mitochondria fuses with the mitochondrial network in the middle. Because the mito-PA-GFP is localized in the matrix, fluorophore transfer indicates full fusion. Images obtained by Zhiyin Song, California Institute of Technology.

Subsequent work has revealed that these behaviors, although at times seemingly random, are critically important for the physiological function of mitochondria. Both mitochondrial fusion and fission are essential functions in mammals, and severe defects in either process result in embryonic lethality in mice. At a cellular level, mitochondrial dynamics has been implicated in multiple functions, including maintenance of mitochondrial morphology, mitochondrial DNA (mtDNA) stability, respiratory capacity, apoptosis, and response to cellular stress. These diverse effects suggest that mitochondrial dynamics plays a central role in the quality control of mitochondria. This review emphasizes advances in our understanding of mitochondrial dynamics in mammals.

THE MACHINERY OF MITOCHONDRIAL FUSION

During mitochondrial fusion, two distinct membrane fusion events occur. The outer and inner membranes, which delineate a mitochondrion, merge with the corresponding membranes on another mitochondrion (**Figure 1*a***). These events result in mixing of the membranes, the intermembrane space, and the matrix. Both membrane fusion events are coordinated and normally occur almost simultaneously, as indicated by live imaging studies showing that content mixing in the matrix can occur rapidly after two mitochondria come in contact with each other (65). Nevertheless, under certain experimental conditions, outer and inner membrane fusion can be temporally separated (76, 80, 110).

In mammals, three large GTPases are essential for mitochondrial fusion (**Figure 2*a***). The mitofusins Mfn1 and Mfn2 are transmembrane GTPases embedded in the mitochondrial outer membrane (98, 100). OPA1 is a dynamin-related GTPase associated with the mitochondrial inner membrane or intermembrane space (1, 25). Depletion of any of these three GTPases results in severely reduced levels of mitochondrial fusion (14, 15, 20, 51, 109).

Mitofusins Mfn1 and Mfn2

The mitofusins were the first proteins found to be important for mitochondrial fusion. The first mitofusin was discovered in *Drosophila* in a mutant characterized by male sterility (53). In early *Drosophila* spermatids, multiple mitochondria are located near the haploid nucleus. As the spermatids mature, the mitochondria aggregate and fuse to form two large mitochondria, which wrap repeatedly around each other to form a spherical structure called the Nebenkern. In cross sections visualized by electron microscopy, the Nebenkern has an onion-slice appearance due to the concentric membranes formed by the wrapped mitochondria. The mitofusin-deficient sterile mutant was named *fuzzy onions* (*Fzo*) because of disruption of the Nebenkern structure. Careful imaging studies indicated that mutation of *Fzo* resulted in failure of the mitochondria to fuse. The function of *Fzo* appears to be restricted to this developmentally regulated fusion of sperm mitochondria. *Drosophila* contains a second mitofusin molecule, Marf/Dmfn, that functions more broadly to mediate fusion of mitochondria in other tissues (27, 60). The yeast ortholog, *Fzo1*, also plays a conserved function in the fusion of mitochondria (57, 97).

Mammals contain two mitofusins, Mfn1 and Mfn2, that localize to the mitochondrial outer membrane and cause aberrations in mitochondrial morphology when overexpressed (72, 100). Mouse knockout studies have provided clear evidence that these proteins are essential for mitochondrial fusion (14, 15). Mouse embryonic fibroblasts (MEFs) lacking either Mfn1 or Mfn2 have highly fragmented mitochondria in contrast to the tubular network observed in wild-type cells. Fusion assays indeed indicate a great reduction in the levels of mitochondrial fusion in single knockout MEFs (15) and a complete loss of fusion in cells lacking both Mfn1 and Mfn2 (14, 69). When mitochondrial fusion rates are reduced, the mitochondrial population fragments into short tubules or small spheres because of ongoing mitochondrial fission in the face of less fusion. These observations support the idea that mitochondrial morphology is dictated by a balance between fusion and fission (4, 15, 104). In addition to mitochondrial fusion, Mfn2 has been implicated in maintaining the sites of contact between the mitochondria and the endoplasmic reticulum (23).

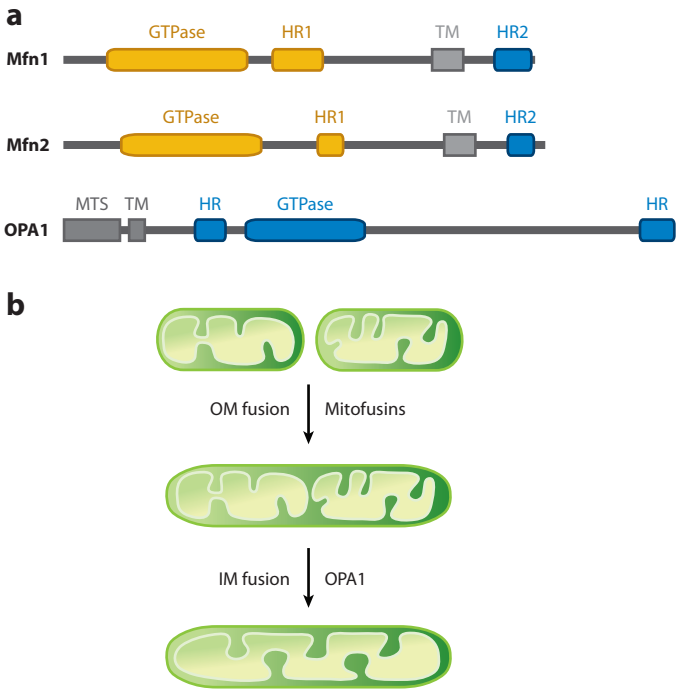

Figure 2

Molecules involved in mitochondrial fusion. (*a*) Schematic of the three central molecules, all large GTPases, necessary for mitochondrial fusion. (*b*) Mitofusins and OPA1 act at sequential steps of mitochondrial fusion. Outer membrane (OM) fusion requires mitofusins, whereas inner membrane (IM) fusion requires OPA1. Abbreviations: HR, heptad repeat; MTS, mitochondrial targeting sequence; TM, transmembrane segment.

OPA1

Human genetic studies identified *OPA1* as the gene mutated in the most common form of dominant optic atrophy, a disease in which retinal ganglion cells degenerate and cause atrophy of the optic nerve (1, 25). This autosomal dominant disease is caused by heterozygous mutations in *OPA1*, leading to the proposal that retinal ganglion cells are vulnerable to mitochondrial dysfunction associated with haploinsufficiency of *OPA1* (26). Because OPA1 is a

Dynamin: a large GTPase that assembles around the necks of endocytic vesicles and uses GTP hydrolysis to generate force for membrane constriction and scission

Cristae: intermembrane space subcompartments that are formed by invaginations of the inner membrane

dynamin-related GTPase that self-assembles, it is also possible that some disease alleles of OPA1 have a dominant-negative mode of action. OPA1 is the mammalian ortholog of Mgm1, which was found to be essential for mitochondrial fusion in yeast (105, 129). Depletion of OPA1 results in severe mitochondrial fragmentation that is due to loss of mitochondrial fusion (14, 20, 51). In MEFs carrying null alleles of *OPA1*, no mitochondrial fusion is detected when matrix mixing assays are used (109, 110).

Along with the loss of mitochondrial fusion, OPA1 deficiency leads to other cellular defects, including reduction and disorganization of cristae membranes (41, 51, 88), severely reduced respiratory capacity (14), and sensitivity to apoptosis (41, 88). In addition to its role in fusion, OPA1 has been proposed to have a direct role in regulating the diameter of cristae junctions during apoptosis (41). Some experimental systems have yielded different views about cristae remodeling during apoptosis (114) and the precise role of OPA1 (131). In yeast, quantitative electron microscopy indicates that Mgm1 is present throughout the inner membrane, with mild enrichment in the inner boundary membrane, the part of the inner membrane that apposes the outer membrane (123). A small, proteolytic fragment of OPA1 has also been implicated in mtDNA replication and distribution (34).

ASSAYS TO MEASURE MITOCHONDRIAL FUSION

Because cells that lack mitochondrial fusion have dramatically fragmented mitochondrial morphology, mitochondrial fragmentation caused by inhibition of protein function is often taken as evidence for a role in mitochondrial fusion. However, it is important to directly measure the levels of mitochondrial fusion because fragmentation can result from either reduced fusion or increased fission. Furthermore, disruption of a protein important for mitochondrial function can secondarily cause a morphological change, particularly fragmentation, even if the protein has no direct or interesting role in mitochondrial fusion. For example, an RNAi screen in *Caenorhabditis elegans* showed that approximately 80% of knockdowns of mitochondrial genes resulted in mitochondrial fragmentation (61). Mitochondrial fragmentation is therefore a common phenotype that must be further evaluated with functional assays.

There are several methods to directly measure mitochondrial fusion in mammalian cells. The first is a cell hybrid assay in which two cell lines containing differentially marked mitochondria are artificially fused with polyethylene glycol. The resulting cell hybrids are assessed for merging of mitochondria (15, 72). The second assay utilizes mitochondrially targeted photoactivatable fluorescent proteins (such as photoactivatable green fluorescent protein) to selectively label a subset of mitochondria within a cell (65, 122). The transfer of this fluorescence to unlabeled mitochondria provides a semiquantitative measure of mitochondrial fusion (**Figure 1*b***). In addition, by targeting the fluorescent protein to the mitochondrial matrix or outer membrane, either full fusion or outer membrane fusion can be selectively analyzed (110). The third method involves in vitro fusion of isolated mitochondria (58, 102), which is similar to a method for analyzing fusion of yeast mitochondria (81).

MECHANISM OF MEMBRANE FUSION

Because mitochondria have double membranes, mitochondrial fusion is necessarily a multistep process. Most assays for mitochondrial fusion (see sidebar, Assays to Measure Mitochondrial Fusion) measure mixing of the matrix contents, a readout that indicates complete fusion. When assessed by such content mixing assays, both *mitofusin*-null and *OPA1*-null cells show complete loss of mitochondrial fusion (14, 110). However, by selectively targeting fluorophores to the mitochondrial outer membrane and matrix, outer membrane fusion in single organelles can be tracked simultaneously with inner membrane fusion. With this dual fluorophore assay, *mitofusin*-null MEFs are devoid of both outer membrane fusion and inner membrane fusion. In contrast, *OPA1*-null MEFs retain outer membrane fusion but lack inner membrane fusion (110). These studies indicate that mitofusins and OPA1 act at distinct steps during the fusion process. Mitofusins mediate outer membrane fusion, and OPA1

subsequently mediates inner membrane fusion (**Figure 2*b***). These assigned functions match the suborganellar localizations of the proteins. Yeast Fzo1 and Mgm1 also play similar, distinct roles in membrane fusion (80). In yeast, Fzo1 and Mgm1 physically interact via Ugo1, an outer membrane protein (105, 130). In mammalian cells, there is no Ugo1 ortholog, but there is evidence for a physical interaction between mitofusins and OPA1 (52). These interactions may serve to mechanistically link outer membrane fusion to inner membrane fusion.

Mitofusins are required on adjacent mitochondria to mediate membrane fusion. In both in vivo and in vitro assays of membrane fusion, mitofusin-deficient mitochondria do not fuse with wild-type mitochondria (69, 81). Mfn1 and Mfn2 form homo-oligomeric and hetero-oligomeric complexes that are competent for fusion (14, 15). The requirement for mitofusins on adjacent mitochondria probably stems from oligomerization of mitofusins during the fusion process. Structural studies indicate that a heptad repeat region in the C-terminal portion of Mfn1 mediates oligomerization through an extended antiparallel coiled coil (69). This structure brings the opposing outer membranes to within approximately 100 Å of each other and thereby tethers mitochondria together during the fusion process. It is likely that subsequent conformational changes, perhaps driven by GTP hydrolysis, are necessary to mediate membrane merger.

Unlike Mfn1 and Mfn2, OPA1 is not required on adjacent membranes for inner membrane fusion to proceed (110). It is remarkable that OPA1-deficient mitochondria are still able to fuse with wild-type mitochondria because the loss of OPA1 results in severe morphological defects in the inner membrane. On the basis of sequence analysis, OPA1 is a dynamin-related GTPase. Consistent with this designation, recombinant OPA1 can interact with membranes and deform their structure (3). OPA1 has a low intrinsic rate of GTP hydrolysis that is enhanced by assembly into a higher-order structure. Although it does not have an obvious pleckstrin homology (PH) domain, OPA1 is able to bind to lipid membranes that contain negatively charged phospholipids. In particular, OPA1 can bind to membranes containing cardiolipin, which is a signature lipid of the mitochondrial inner membrane. Assembly on lipid membranes causes an increase in GTP hydrolysis. In both OPA1 and Mgm1, mutations in the region roughly corresponding to the PH domain in dynamin can disrupt lipid binding (3, 99). Upon binding to liposomes, OPA1 can deform the surface and cause the elaboration of lipid tubules (3). Mutations that are involved in dominant optic atrophy have been shown to disrupt GTP hydrolysis or lipid binding by OPA1.

Because of differential RNA splicing (24) and protein processing, OPA1 exists as a mixture of protein isoforms. OPA1 can be processed from a precursor into a long isoform that is membrane-anchored or a short isoform that lacks the membrane anchor but can still interact with membranes. Under normal conditions, a combination of both long and short isoforms is required for membrane fusion (109). However, during the response of cells to stress, the long isoform alone appears to be sufficient to enhance mitochondrial fusion (120). How the long and short isoforms of OPA1 work together to mediate fusion is unknown. In yeast, coassembly of the long and short isoforms of Mgm1 results in enhanced GTP hydrolysis (28).

THE MACHINERY OF MITOCHONDRIAL FISSION

The balance between the opposing processes of fusion and fission maintains the overall morphology of mitochondria and ensures that the mitochondrial population remains dynamic. Genetic and cell biological studies have identified two classes of molecules that are necessary for the fission of mitochondria. The central player appears to be Drp1, a dynamin-related protein. Analogous to the role of dynamin in endocytic vesicle fission (103), Drp1 assembles on mitochondrial tubules and is thought to mediate constriction and scission (4, 70, 104, 108). Much of Drp1 resides in the cytosol, and

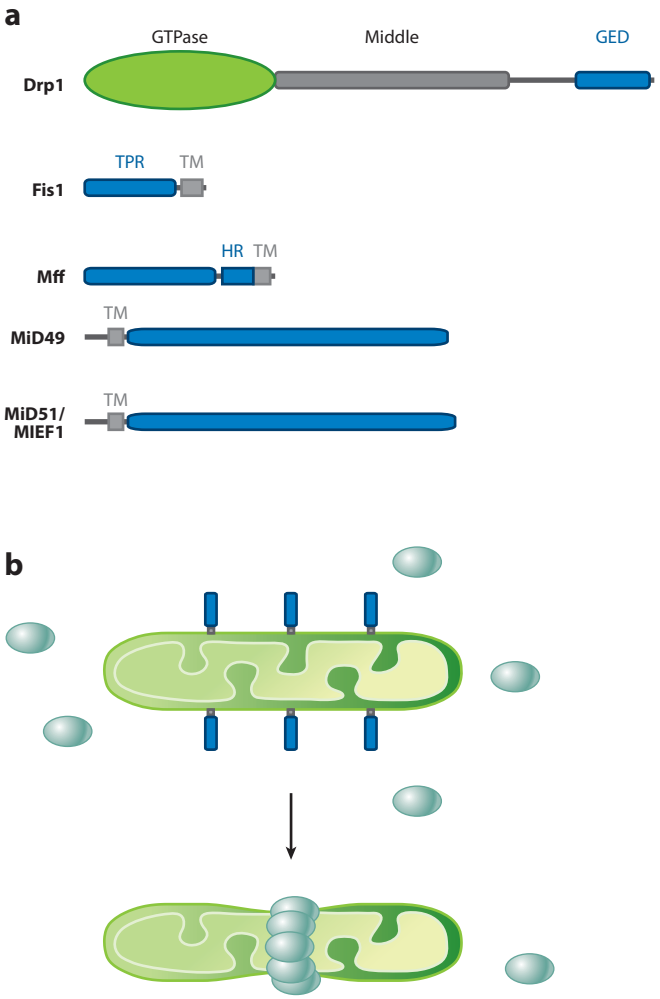

Figure 3

Molecules involved in mitochondrial fission. (*a*) Schematic of Drp1 and the four potential Drp1 receptors: Fis1, Mff, MiD49, and MiD51/MIEF1. Drp1, as with other dynamin-related proteins, has a GTPase, middle, and GED (GTPase effector domain) domain. All four potential Drp1 receptors have a single transmembrane segment (TM) anchored in the outer membrane, with a membrane topology such that most of the protein (*blue regions*) protrudes into the cytosol. Only the cytosolic region of Fis1 is well characterized. It forms a six-helix bundle containing a pair of tetratricopeptide (TPR)-like motifs. (*b*) Recruitment of Drp1. Drp1 is cytosolic unless recruited by molecules on the mitochondrial surface. The candidate Drp1 receptors are depicted generically. The initial stage of mitochondrial constriction (not depicted) is Drp1 independent. After recruitment, Drp1 assembles into a higher-order structure that further constricts the mitochondrial tubule. Abbreviation: HR, heptad repeat.

therefore a second class of proteins on the mitochondrial surface is necessary to efficiently recruit Drp1 for fission (**Figure 3*a*,*b***). This second class potentially includes Fis1, Mff, MiD49, and MiD51.

However, some observations suggest that our inventory of mitochondrial fission proteins is incomplete. First, imaging studies suggest that constriction sites on mitochondrial tubules are often associated with the endoplasmic reticulum (ER) (42). This mitochondria-ER association is found prior to Drp1 recruitment, suggesting that it may be an early event that marks fission sites. The molecules underlying this phenomenon remain to be discovered. Second, electron microscopy studies indicate that the mitochondrial matrix can sometimes divide in the absence of outer membrane fission (43). Because the known fission machinery assembles on the mitochondrial outer membrane, the molecules mediating this inner membrane fission process also remain to be discovered.

Drp1

The dynamin-related protein Drp1 is essential for most types of mitochondrial fission. Inhibition of Drp1 function, either by expression of a dominant-negative variant or RNA interference, results in very elongated mitochondria that entangle and collapse (71, 108). MEFs containing null alleles of Drp1 similarly show elongated mitochondria (64, 124). Interestingly, whereas Drp1 mutant MEFs are resistant to mitochondrial fragmentation induced by carbonyl cyanide *m*-chlorophenylhydrazone (CCCP, an ionophore that dissipates the mitochondrial membrane potential), they show substantial mitochondrial fragmentation in response to apoptotic stimuli such as actinomycin D and etoposide (64, 124). These observations suggest that some types of mitochondrial fission can occur in the absence of Drp1.

The role of Drp1 in mediating mitochondrial fission is thought to be similar to that of dynamin in scission of endocytic vesicles. Dynamin is the prototypical member of the dynamin family of GTPases (38).

During endocytosis, dynamin assembles into higher-order oligomers that encircle the necks of endocytic vesicles. The interaction of dynamin with membranes is mediated by the PH domain, a lipid-binding structure. Nucleotide-dependent conformational changes cause rearrangement of the lipids within the membrane bilayer, leading to a hemifission intermediate followed by full scission (103). Recent structural studies provide important insights into how conformational changes in dynamin might result in membrane deformation (12, 36, 39).

Given the high sequence similarity between Drp1 and dynamin, Drp1 likely also constricts mitochondrial tubules in a similar manner to mediate membrane fission. Fluorescence microscopy indicates that Drp1 can be recruited from the cytosol to mitochondria, where it forms puncta encircling the tubule (40, 70). The sites of Drp1 recruitment are often constricted, and only a subset of puncta progress to a productive fission event. Drp1, as well as the yeast Dnm1, can assemble in vitro into higher-order helical structures that may correspond to the Drp1 puncta found on mitochondria (62, 79, 108, 135). Unlike dynamin, Drp1 does not have an obvious PH domain. Drp1 is recruited to the mitochondrial membrane by receptor-like molecules on the mitochondrial outer membrane (see below). It is currently unknown whether Drp1, once recruited, subsequently interacts directly with the membrane. Dnm1 can assemble on artificial liposomes in vitro and cause a large constriction upon GTP hydrolysis (79).

Given the central role of Drp1 in the fission of mitochondria, it is important to understand how Drp1 is recruited from the cytosol onto the mitochondrial surface. Below, the four strongest candidates for Drp1 recruitment are reviewed.

FIS1

In the budding yeast *Saccharomyces cerevisiae*, there are two clear requirements for recruitment of Dnm1 (the yeast Drp1 ortholog). First, Dnm1 recruitment requires the outer membrane protein Fis1. Loss of Fis1 results in a severe fission defect due to failure to recruit Dnm1 from the cytosol (37, 85, 118). Second, Dnm1 recruitment requires one of two WD40-containing adaptors, Mdv1 or Caf4 (49). These adaptors contain an N-terminal half that dimerizes and binds to Fis1 and a C-terminal half containing WD40 repeats that binds to Dnm1 (7, 49, 119). In this way, Mdv1 and Caf4 bridge the interaction of Fis1 with Dnm1. However, there is also a report that Fis1 can directly bind Dnm1 (128). Mdv1 is the physiologically more important adaptor because loss of Mdv1 results in a severe mitochondrial fission defect, whereas loss of Caf4 does not (49). Although the process of Dnm1 recruitment in yeast is relatively well defined, the situation is much less clear in mammalian cells. Orthologs to the yeast adaptors Mdv1 and Caf4 do not exist in mammalian cells.

Moreover, despite strong anticipation that mammalian Fis1 would recruit Drp1, there is conflicting evidence for such a role. Early studies supported such a function because overexpression of Fis1 in mammalian cells results in fragmentation of mitochondria (111, 134, 137). More importantly, inhibition of Fis1 by antibody injection (134), antisense oligonucleotides (134), or RNA interference (45, 68, 71, 111) results in elongation of mitochondria. These results provide good evidence for an important role of Fis1 in mitochondrial fission. Perplexingly, though, Fis1 inhibition does not abrogate the recruitment of Drp1 onto the mitochondrial surface (71). A more recent study has questioned the role of Fis1 in mitochondrial fission (89). Knockdown of Fis1 in HeLa cells does not significantly alter mitochondrial morphology. In addition, targeted deletion of the *Fis1* gene from human carcinoma HCT116 cells does not disrupt mitochondrial morphology or Drp1 recruitment to mitochondria (89). It is difficult to synthesize these results, but one possibility is that Fis1 may play an essential role in mitochondrial fission in only specific cell types. In other cell types, other molecules, such as Mff, may play the dominant role.

Mitochondrial membrane potential: the electrical gradient across the mitochondrial inner membrane driven by pumping of protons outward by the electron transport chain

Mff

The mitochondrial outer membrane protein Mff is the best established receptor for Drp1. *Mff* was identified in a small interfering RNA screen for *Drosophila* genes whose knockdown results in mitochondrial elongation (45). Knockdown of the corresponding mammalian gene similarly causes mitochondrial elongation. Like Fis1, Mff is a tail-anchored protein of the mitochondrial outer membrane. Analysis by blue native gel electrophoresis indicates that Mff and Fis1 exist in separate large-molecular-weight complexes.

Importantly, knockdown of Mff reduces the amount of Drp1 that is recruited to mitochondria (89), thereby implicating Mff as a receptor for Drp1. In immunoprecipitation experiments with cross-linker, Mff and Drp1 physically associate with each other. In contrast, knockdown of Fis1 fails to disrupt Drp1 recruitment to mitochondria (71, 89). Taken together, these observations suggest that Mff acts as a receptor for Drp1 in HeLa cells. It remains possible that Fis1 plays an important role in fission in selected cell types.

Interestingly, the machinery for fission appears to be shared between mitochondria and peroxisomes. Drp1, Fis1, and Mff all localize, in part, to peroxisomes, and their depletion has been reported to cause elongation of peroxisomes (45, 68, 74, 124, 127). There is at present no evidence that peroxisomes undergo fusion.

MiD49 and MiD51/MIEF1

MiD49 (mitochondrial dynamics protein of 49 kDa) and MiD51/MIEF1 (mitochondrial dynamics protein of 51 kDa/mitochondrial elongation factor 1) are similar proteins located on the mitochondrial outer membrane. Each contains a single-pass transmembrane segment near the N terminus, with the bulk of the protein protruding into the cytosol. Overexpression of either protein in mammalian cells causes the mitochondrial network to become excessively elongated or to collapse (90, 139), a phenotype reminiscent of a fission defect. In such cells, increased recruitment of Drp1 puncta to the mitochondria is observed, suggesting that either MiD49 or MiD51 is capable of Drp1 recruitment. Indeed, both two-hybrid and coimmunoprecipitation experiments indicate that these proteins can physically interact with Drp1 (90).

The exact roles of the MiD proteins in mitochondrial dynamics are currently somewhat unclear. The two studies on MiD function report different effects of MiD knockdown. In one study (90), knockdown of either MiD49 or MiD51/MIEF1 had no effect on mitochondrial morphology, but simultaneous knockdown of both resulted in elongated mitochondria. The latter result indicates that the MiD proteins function to promote mitochondrial fission. To explain the overexpression result, it has been hypothesized that although excessive MiD production results in increased Drp1 recruitment, the recruited Drp1 is not active, thereby resulting in a fission defect. A second study (139), however, found that knockdown of MiD51/MIEF1 alone resulted in mitochondrial fragmentation, leading to the conclusion that MiD51/MIEF1 recruits Drp1 but acts to inhibit its fission activity. The available evidence therefore indicates that the MiD proteins are important regulators of Drp1 function, but more studies are necessary to understand their precise roles.

REGULATION OF MITOCHONDRIAL DYNAMICS

Proteolytic Cleavage of OPA1

With its central role in inner membrane fusion, OPA1 is regulated through multiple mechanisms. The *OPA1* gene encodes eight distinct mRNA splice forms that are regulated by differential splicing (24). Each mRNA splice form encodes a precursor protein containing several important features at the N terminus. The extreme N terminus contains a mitochondrial targeting sequence, followed by a transmembrane segment, and then a protease cleavage site designated as S1. In four of the eight mRNA

splice forms, there is an additional S2 protease cleavage site. As a result, each mRNA splice form has the potential to produce two or more protein isoforms (**Figure 4*a***). Upon import of the N terminus into the mitochondrial matrix, the mitochondrial targeting signal is cleaved by the mitochondrial processing peptidase to produce a long isoform that is anchored to the mitochondrial inner membrane via the transmembrane segment. However, the precursor protein can also be cleaved at either S1 or S2 to yield a short isoform that lacks the transmembrane segment (**Figure 4*b***). Except for the special case of stress-induced hyperfusion (120), individual long or short isoforms are not competent for fusion; a combination of both is required (109). Although lacking the transmembrane anchor, short isoform 1 can nevertheless associate with lipid membranes through a different mechanism (3).

Because mitochondrial fusion depends on a proper ratio of long to short OPA1 isoforms, the proteolytic processing of OPA1 at S1 and S2 is a major source of regulation. OPA1 processing is enhanced by several types of mitochondrial dysfunction, including loss of membrane potential, apoptosis, mtDNA disease, and high loads of mtDNA mutations (31, 63). Under these conditions, the short isoforms of OPA1 predominate, and fusion activity is diminished. Early studies focused on the mitochondrial rhomboid protease PARL (presenilin-associated rhomboid-like) as the processing protease for OPA1 (21), given that yeast Mgm1 is processed by a mitochondrial rhomboid protease (56, 78). However, loss of mammalian PARL has little or no detectable effect on OPA1 processing and mitochondrial morphology (21, 32).

The mitochondrial proteases Yme1L and OMA1 have emerged as the most likely candidates for mediating the proteolytic processing of OPA1. Knockdown of the i-AAA protease Yme1L blocks cleavage of OPA1 at the S2 site and alters the distribution of OPA1 isoforms (50, 109). OMA1 (named for overlapping activity with m-AAA protease), a zinc metalloprotease localized on the inner membrane,

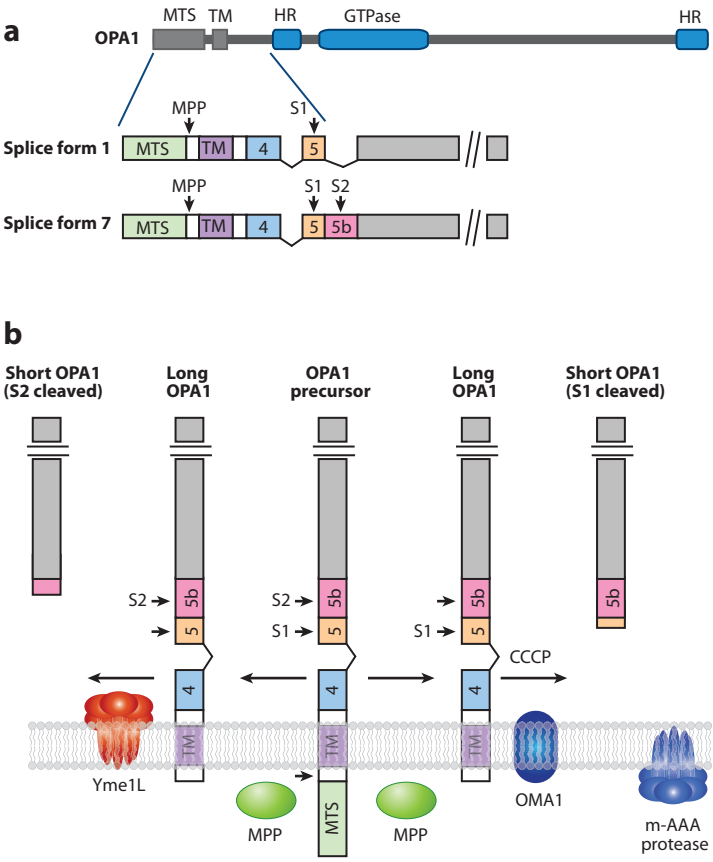

Figure 4

Regulation of OPA1. (*a*) Differential splicing. The OPA1 gene encodes eight RNA splice forms; for simplicity, only splice forms 1 and 7 are illustrated. Splice form 1 encodes a polypeptide with an S1 protease cleavage site. Splice form 7 contains an extra exon (exon 5b) and thereby encodes a polypeptide with both S1 and S2 protease cleavage sites. MPP is the site of cleavage by the mitochondrial processing protease (MPP). (*b*) Protease processing of OPA1. In the center, the precursor polypeptide encoded by splice form 7 is shown. After import of the amino terminus into the matrix, the mitochondrial targeting sequence is cleaved by the MPP to yield the membrane-anchored, long isoform of OPA1. Further processing by the Yme1L protease at the S2 cleavage site yields a short isoform of OPA1 (*left side*). Cleavage at the S1 site also yields a short isoform. When mitochondria are depolarized by CCCP (carbonyl cyanide *m*-chlorophenylhydrazone), the inner membrane OMA1 protease is activated to cleave at the S1 site (*right side*). The m-AAA protease can also regulate OPA1 processing indirectly through OMA1. Abbreviations: HR, heptad repeat; MTS, mitochondrial targeting sequence; TM, transmembrane segment.

is necessary for S1 cleavage of OPA1 that is induced by loss of membrane potential (33, 55). OMA1 itself is normally degraded by an unknown protease but accumulates in depolarized mitochondria (55). The dependence of OPA1

processing on Yme1L and OMA1 is clear, but it remains to be determined if they directly cleave OPA1. In addition, when the m-AAA proteases AFG3L1 and AFG3L2 are knocked down in mouse cells, OPA1 is again inhibited by excessive processing by OMA1 (33). Therefore, a complicated proteolytic network regulates the processing of OPA1. Moreover, the inner membrane prohibitins also play a role in regulating OPA1 processing. Cells lacking prohibitin 2 (PHB2) show selective loss of long isoforms of OPA1 and a strong defect in mitochondrial fusion (82).

Regulation of Drp1

Multiple posttranslational modifications have been implicated in the regulation of Drp1 function, including phosphorylation, S-nitrosylation, ubiquitination, and sumoylation. These modifications have been reviewed previously (10), and only phosphorylation is summarized here to highlight some notable recent advances.

Two main phosphorylation sites have been established in Drp1, at amino acids S616 and S637 (numbering corresponds to human Drp1, isoform 1) (9, 22, 115). Cdk1/cyclin B phosphorylates the S616 site during mitosis and results in activation of fission activity (115). The mitotic kinase Aurora A is also involved in this mitotic activation of Drp1 (66). It phosphorylates the Ras-like GTPase RALA, thereby enriching RALA and its effector RALBP1 on the mitochondria. RALBP1 enhances phosophorylation of Drp1 by Cdk1/cyclin B. Mitochondria show cell cycle–dependent changes in morphology, ranging from an elongated network at the G1/S transition to fragmented spheres at mitosis (83). Activation of Drp1 during mitosis is responsible for this mitotic mitochondrial fragmentation (115), which is thought to facilitate the equal distribution of mitochondria to daughter cells.

Phosphorylation at S637 is increased in response to various stimuli, including forskolin, isoproterenol, and forced exercise (22). The effect of S637 phosphorylation on Drp1 function seems to depend on the cellular context. Phosphorylation by protein kinase A (PKA, also called cAMP-dependent protein kinase) leads to inhibition of fission activity (9, 22). S637 is located in the GTPase effector domain, and phosphorylation impairs the interaction of the GED with the middle domain and also reduces the GTP hydrolysis activity of Drp1 (9). This phosphorylation can be reversed by the phosphatases calcineurin (6, 19, 22) or PP2A/Bβ2 (29). In rat hippocampal neurons treated with potassium, a rise in intracellular calcium activates phosphorylation of Drp1 at S637 by Ca^{+}/calmodulin-dependent protein kinase α (CaMKIα) (54). In this case, however, phosphorylation activates mitochondrial fission and causes fragmentation. The possible reasons for the different effects of phosphorylation at S637 have been discussed elsewhere (10) and likely reflect the different cellular contexts used to study Drp1. Phosphorylation of Drp1 also regulates mitochondrial fission during starvation conditions (discussed below).

PHYSIOLOGICAL FUNCTIONS OF MITOCHONDRIAL DYNAMICS

When the molecules essential for mitochondrial fusion and fission were first discovered, many observers asked why mitochondria were dynamic in the first place. It soon became clear that the balance between fusion and fission serves to maintain the characteristic morphology of mitochondria in a given cell type (4, 15, 104). If mitochondrial fusion is reduced, the mitochondria become fragmented because of unbalanced fission. Conversely, if mitochondrial fission is reduced, the mitochondria become elongated and excessively interconnected because of unbalanced fusion. Therefore, these opposing processes work in concert to maintain the shape, size, and number of mitochondria. Over the past decade, however, it has become clear that the functions of fusion and fission go far beyond the appearance of mitochondria and indeed have important physiological consequences. Mice lacking molecules important for mitochondrial fusion or fission cannot survive

past midgestation and show cell type–specific defects. Insights from these and other studies are summarized below.

Content Mixing

Perhaps the most important consequence of mitochondrial fusion is content mixing between mitochondria. After two mitochondria fuse, there is mixing of the outer membranes, the inner membranes, and the matrix contents. From the comparative analysis of *mitofusin*-null and *OPA1*-null cells, fusion of the inner membrane—and therefore the matrix contents—appears to be critical. The mitochondrial defects of OPA1-deficient cells are as severe as those of mitofusin-deficient cells. In other words, even though some outer membrane fusion is preserved in OPA1-deficient cells, this partial fusion activity is not sufficient to preserve mitochondrial function, suggesting that matrix mixing is a primary role of mitochondrial fusion (110). Mitochondrial fusion assays using photoactivatable green fluorescent protein indicate that when mitochondria touch each other, content mixing often occurs even without prolonged merging between the two organelles (75). This type of fusion event does not change the morphology of the mitochondria involved and has been termed "kiss-and-run." The high frequency of such content exchange events implies that they are functionally important.

How might content exchange benefit a mitochondrial population? A crude model can be proposed, although a precise answer requires a quantitative understanding of mitochondrial biogenesis at the systems biology level. Mitochondria are complex organelles with a dual genetic blueprint. Of the approximately 1,000 or more proteins present in the mitochondrial proteome, 13 polypeptide chains are encoded by the mitochondrial genome. The rest are encoded by the nuclear genome and must be imported from the cytosol. During mitochondrial biogenesis, the levels of these two sets of proteins must be coordinately regulated. If mitochondrial fusion did not exist, each of the several hundred mitochondria in a typical cell would act autonomously, and their biochemical and functional profiles would diverge. There is indeed increased heterogeneity within the mitochondrial populations of mitofusin-null and OPA1-null cells (14). With mitochondrial fusion, the contents of the organelles are intermittently homogenized, and therefore the mitochondria can act as a coherent population. When mitochondrial fusion is disrupted, the loss of content mixing may be responsible for many of the functional defects described below, such as mtDNA instability or reduced respiratory capacity.

The scenario above could operate in normal, healthy cells to maintain genetic and biochemical uniformity within the mitochondrial population. It can also be imagined that mitochondrial fusion plays a protective role in certain pathological conditions. By mixing mitochondrial contents, fusion can protect function by allowing complementation of mtDNA mutations. Most mtDNA mutations involved in mitochondrial encephalomyopathies are recessive, and mammalian cells can tolerate a surprisingly high load of mtDNA mutations without a reduction in respiratory capacity (30, 86). Mitochondrial fusion, along with the high copy number of mtDNA genomes, would allow cells to carry a higher mutational load without detriment to respiratory function. In support of this idea, mice carrying an error-prone mtDNA polymerase cannot tolerate a reduction in mitochondrial fusion (17). Combining mutations in mtDNA polymerase and *Mfn1* leads to a neonatal synthetic lethality. Cells carrying both mutations show severe respiratory loss in contrast to the normal respiratory function of cells carrying single mutations.

Although mouse knockout and human disease studies clearly indicate the important impact of mitochondrial fusion, this process has to be carefully regulated to prevent undesired effects. Fusion is highly dependent on the mitochondrial membrane potential. When mitochondria divide, a daughter mitochondrion that loses membrane potential fails to

Photoactivatable green fluorescent protein: a green fluorescent protein variant whose fluorescent intensity is greatly increased by illumination with short wavelength light (~405 nm)

Mitochondrial encephalomyopathies: a diverse group of diseases that are caused by mutations in mtDNA and typically include muscle and nervous system symptoms

Heteroplasmic: describes a state in which cells contain more than one type of mtDNA genome, such as a wild-type genome and a mutant genome

Mitochondrial outer membrane permeabilization (MOMP): an early apoptotic event in which pores form on the mitochondrial outer membrane to allow exit of proapoptotic molecules from the inner membrane space

fuse back into the mitochondrial population (121). In part, the inhibition of fusion is due to increased proteolytic processing of OPA1. These observations have led to the model that mitochondria with loss of membrane potential are segregated from the rest of the mitochondrial population and subsequently degraded by autophagy (121). Therefore, the fate of a dysfunctional mitochondrion likely depends on the degree of dysfunction as well as the type of dysfunction. Mitochondria that are moderately dysfunctional can be rescued by mitochondrial fusion, whereas those with severe dysfunction may be segregated and degraded.

Although there is no direct evidence, it can also be speculated that mitochondrial fusion is involved in certain pathological conditions. Cells that are heteroplasmic for certain mtDNA mutations can become homoplasmic when cultured, indicating expansion of mutant mtDNA molecules at the expense of wild-type ones (133). In addition, as humans age, there is a propensity for certain cell types to show clonal expansion of a dysfunctional mtDNA genome containing a deletion. This trend is especially well documented in aged skeletal muscle, where respiratory-deficient fibers often contain high levels of a mutant mtDNA genome (125). The molecular bases of these phenomena are poorly understood, but it is possible that mitochondrial fusion in such circumstances is detrimental because it allows persistence and spread of the mutant mtDNA molecules.

mtDNA Stability

Cells with loss of mitochondrial fusion show a dramatic reduction in the level of mtDNA. MEFs lacking OPA1 or both Mfn1 and Mfn2 contain only a third of the normal levels of mtDNA (17). Along with reduced overall mtDNA levels, there is also a defect in the number and distribution of mtDNA nucleoids such that a subpopulation of mitochondria does not contain any mtDNA (16). Because the mtDNA genome encodes for 13 essential subunits of the respiratory chain, mitochondria lacking nucleoids are respiratory deficient.

This dependence of mtDNA maintenance on mitochondrial fusion is also found in mouse skeletal muscle. During the first 2 months of life, the levels of mtDNA in skeletal muscle undergo a dramatic increase relative to nuclear DNA. In mice lacking Mfn1 and Mfn2 in skeletal muscle, this developmental increase in mtDNA fails to occur, leading to a reduction in mtDNA levels down to less than 10% of wild-type muscle (17). It is likely that this reduction of mtDNA levels is in part responsible for the severe respiratory deficiency observed in such muscles. In addition to reduced mtDNA levels, skeletal muscles lacking Mfn1 and Mfn2 also show an increased accumulation of both point mutations and deletions (17). Yeast cells without Fzo1 or Mgm1 are ρ^0 (devoid of mtDNA) and respiratory deficient (57, 97, 129).

Respiratory Function

MEFs lacking OPA1 or both Mfn1 and Mfn2 have reduced respiratory capacity; *OPA1*-null cells have a more pronounced defect (14). OPA1-null cells also have a more severe defect in cristae structure, but whether this contributes further to the respiratory decline is not known. Beyond cell lines, cerebellar Purkinje cells (16), skeletal myocytes (17), and cardiac myocytes (18) all show respiratory decline upon deletion of mitofusins. In the case of Purkinje cells and skeletal muscle, there is a compensatory but futile increase in mitochondrial content (16, 17).

Facilitation of Apoptosis by Mitochondrial Fission

The intrinsic pathway of apoptosis critically depends on the release of cytochrome *c* and other proapoptotic molecules from the mitochondrial inner membrane space into the cytosol (116). This release of cytochrome *c* happens when mitochondrial outer membrane permeabilization (MOMP) occurs because of

the actions of the proapoptotic Bcl-2 family members Bax and Bak. Once released into the cytosol, cytochrome *c* binds to apoptotic protease-activating factor 1 to form a complex that cleaves and activates the initiator caspase 9. Caspase 9 then activates the executioner caspases 3 and 7, leading to the degradation of many substrates.

In cellular models of apoptosis, mitochondria undergo increased fission and fragment near the time of cytochrome *c* release (40). Remarkably, inhibition of Drp1 function can prevent this induced mitochondrial fission, and furthermore, reduce cytochrome *c* release and the level of cell death (40). The involvement of Drp1 in apoptosis has been confirmed in a number of other studies (5, 47, 71), leading to the idea that Drp1 might work with Bax and Bak to permeabilize the mitochondrial outer membrane (112). In vitro, Drp1 has been shown to enhance the ability of Bax to assemble on lipid membranes (84). This observation led to the hypothesis that mitochondrial membrane deformations mediated by Drp1 can facilitate Bax oligomerization during apoptosis. Apoptosis is also alleviated in cells with reduced Mff or Fis1 (45, 71). In contrast, mitochondrial fusion is protective against some apoptotic stimuli (113, 120), although exceptions have been reported (106).

However, whether mitochondrial fission plays a central role in MOMP remains widely debated (77, 107). In some studies, inhibition of Drp1 merely delays cytochrome *c* release and does not ultimately reduce the extent of cell death (35, 91). Moreover, even though cytochrome *c* release is delayed, release of other proapoptotic molecules, such as SMAC/DIABLO, is not affected (35, 64, 91). Studies of Drp1 knockout mice have also provided contrasting evidence for the role of Drp1 in apoptosis. Cells devoid of Drp1 have no defect in apoptosis (124) or only a mild delay in the kinetics of cytochrome *c* release (64). These data indicate that MOMP by Bax and Bak can largely proceed in the absence of Drp1. In fact, in mouse tissues, loss of Drp1 causes some cells to undergo increased apoptosis. For example, there is a widespread increase in apoptotic neurons in the embryonic and adult brain of Drp1 mutant mice (64). In addition, primary cultured neurons from these mice are more sensitive to apoptosis when treated with staurosporine, a protein kinase inhibitor that is a common apoptosis inducer (64). Paradoxically, one study hinted that Drp1 mutant embryos have fewer apoptotic cells in the neural tube, a site of developmentally regulated apoptosis (124). Clearly, more studies are necessary to resolve the relationship of mitochondrial fission to apoptosis. At this point, it appears that inhibition of Drp1 can have a moderately protective effect in some models of apoptosis in contrast to the large effects found with expression of Bcl-2 or caspase inhibitors.

Adaptation to Cellular Stress

Several recent studies indicate that changes in mitochondrial dynamics can allow cells to adapt to certain types of stress. Many cell lines show elongated mitochondria in response to UV light irradiation, general inhibition of RNA transcription, general inhibition of protein translation, and nutrient starvation. This response has been termed stress-induced mitochondrial hyperfusion (SIMH) (120) and depends on Mfn1, OPA1, and stomatin-like protein 2. SIMH results in increased ATP production and is thought to be a prosurvival response to stress. Mutant cells unable to mount such a response show increased vulnerability to stress and more readily undergo apoptosis.

Two other studies have highlighted the role of mitochondrial fission in adaptation of cell lines to serum and amino acid starvation (48, 96). During starvation, autophagy is activated to degrade cellular components for recycling, but mitochondria instead elongate and escape autophagy. In contrast to SIMH, which is driven by increased fusion, the elongation observed here is due to inhibition of fission. There is less Drp1 localized to mitochondria, a reduction in the stimulatory phosphorylation on residue S616, and an increase in inhibitory phosphorylation on S637. Mutant cells that

Mitophagy: disposal of mitochondria via the autophagy pathway

are unable to undergo the elongation response show increased mitophagy during nutrient deprivation.

Mitophagy is a quality control process in which dysfunctional mitochondria are selectively degraded by the autophagy (67). In mouse hepatocytes, mitochondria that have lost membrane potential are recognized by the autophagy pathway. The serine/threonine kinase PINK1 and the E3 ubiquitin ligase Parkin have been implicated in a pathway to degrade dysfunctional mitochondria (87, 136). These two genes are found mutated in some cases of familial Parkinson's disease (PD) and provide evidence that mitochondrial dysfunction is an underlying feature of PD. PINK1 accumulates on mitochondria that have lost membrane potential and recruits Parkin to the mitochondrial surface (136). Once recruited to the mitochondrial surface, Parkin polyubiquitinates many proteins on the mitochondrial outer membrane, resulting in their degradation via the ubiquitin-proteasome system (8, 117). Among the most rapidly degraded proteins are Mfn1, Mfn2, and two proteins involved in mitochondrial trafficking, Miro1 and Miro2 (8, 46, 95, 117). It is thought that degradation of these proteins inhibits both mitochondrial fusion and transport, thereby segregating the dysfunctional mitochondria from the rest of the population. In the case of Miro, phosphorylation by PINK1 is important for proteasomal degradation (126). In addition, Drp1 is recruited to mitochondria and appears important for the subsequent mitophagy (117). The requirement for mitochondrial fission is thought to reflect the need to reduce the size of mitochondria so that they are suitable substrates for engulfment by the autophagosome. The influence of mitochondrial fusion and fission on Parkin-mediated mitophagy may underlie the strong genetic interactions between PINK1/Parkin and Mfn/OPA1/Drp1 in *Drosophila* (27, 94, 132). The role of mitochondrial fusion and fission in mitophagy is not limited to Parkin-mediated mitophagy and has been found in other mitophagy models (121).

MITOCHONDRIAL DYNAMICS IN DISEASE

Given the importance of fusion and fission in mitochondrial physiology, it is perhaps not surprising that there are human diseases caused by mutations in mitochondrial dynamics genes. The discussion here is restricted to diseases directly caused by mutations in core proteins involved in mitochondrial dynamics; however, mitochondrial dynamics appears to be involved in a much broader set of diseases (13).

OPA1 and Autosomal Dominant Optic Atrophy

Heterozygous mutations in *OPA1* cause autosomal dominant optic atrophy (DOA), Kjer's type (1, 25). In the classic presentation, disease onset occurs in childhood or early adulthood with slowly progressive visual loss. Fundoscopic exam reveals temporal pallor that is indicative of atrophy of the optic nerve. Pathological analysis indicates that DOA is caused by degeneration of the retinal ganglion cells, whose axons are bundled to form the optic nerve. The optic nerve transmits visual information from the retina to other parts of the brain.

Although the classical presentation of DOA indicates that retinal ganglion cells are particularly vulnerable to a reduction in OPA1 function, more recent clinical studies indicate that a wider range of cell types can be affected by this disease. A large-scale study of DOA patients with *OPA1* mutations estimates that as many as one in six patients have extraocular clinical features (138), a presentation termed OPA1+. The most common extraocular feature is neurosensory hearing loss, but a wide range of other features has been described, including ataxia, myopathy, and peripheral neuropathy. Interestingly, myopathy is associated with the presence of respiratory-deficient muscle fibers that contain multiple mtDNA deletions, implying that OPA1, like mitofusins, is important for mtDNA stability (2, 59). In addition, the presence of peripheral neuropathy in some patients is notable because Mfn2

mutations cause peripheral neuropathy in Charcot-Marie-Tooth type 2A (CMT2A).

Mfn2 and CMT2A

Heterozygous mutations in *Mfn2* cause CMT2A (141), an autosomal dominant disease characterized by axonal peripheral neuropathy. Patients have weakness, muscle atrophy, and sensory loss in the distal limbs. The age of onset is variable, and the disease is slowly progressive. Although CMT2A disease is clinically similar to CMT1, the latter disease is characterized by demyelination of the peripheral nerves and therefore slow motor nerve conduction velocity.

Classic CMT2A is limited to the peripheral nerves, but as with DOA, sporadic families manifest additional features. In addition, a related clinical entity termed hereditary motor and sensory neuropathy VI is also caused by heterozygous mutations in *Mfn2* (140). Patients with this disease have optic atrophy in addition to peripheral neuropathy. Although the classical forms of DOA and CMT2A highlight the tissue-specific effects of *OPA1* and *Mfn2* mutations, it is now clear that there can be substantial overlap in the clinical spectrum of these diseases. Mutations in either *OPA1* or *Mfn2* can cause defects in the optic nerve as well as in the peripheral nerves. Both DOA and CMT2A can be characterized as mitochondrial diseases, which often have variable and broad clinical presentations.

Except for some rare reports of compound heterozygosity (92, 93, 101), almost all cases of disease associated with OPA1 or Mfn2 involve heterozygous mutations and a dominant inheritance pattern. Based on mouse knockout studies, it is probable that severe homozygous mutations would lead to early lethality. Consistent with this idea, an autosomal recessive neurodegenerative disease in dogs termed neuroaxonal dystrophy is caused by a homozygous *Mfn2* mutation that removes a single codon and results in little or no Mfn2 protein (44). The affected dogs die at birth due to pulmonary failure. The clinical findings are widespread and include poor motor function, hypoplasia of the cerebellum and lungs, and segmental swelling of axons throughout the nervous system.

Drp1 and Neonatal Lethality

Only a single case study of a patient with a *Drp1* mutation has been described (127). This patient was a newborn girl who had multisystem abnormalities, including small head circumference, hypotonia, few spontaneous movements, optic atrophy, and poor feeding. She died at 37 days. Analysis of fibroblasts indicated elongated mitochondria and peroxisomes. The heterozygous mutation (A395D) in this patient resides in the middle domain (**Figure 3**), which in dynamin-family proteins is involved in self-assembly. Overexpression of this allele in wild-type cells induced mitochondrial and peroxisomal abnormalities, suggesting a dominant-negative mode of action. Biochemical and two-hybrid analysis suggest that this mutant dramatically disrupts higher-order assembly of Drp1 and thereby reduces assembly-stimulated GTP hydrolysis (11).

CONCLUSION

It is now clear that mitochondrial fusion and fission are fundamental processes necessary for the health of mitochondria. Mitochondrial dynamics is probably a ubiquitous phenomenon, but it seems likely that different cell types harness these processes in distinct ways suited to their cellular physiology. Most studies of mitochondrial dynamics have relied on convenient cell culture systems, and we still have much to learn about the behavior of mitochondria in intact tissues. At the other end of the spectrum, more work is needed to understand the molecular mechanics of mitochondrial dynamics. How do mitofusins and OPA1 mediate the merger of lipid membranes, and how is outer membrane fusion mechanistically coupled to inner membrane fusion? How is Drp1 recruited to mitochondria, and what molecules are needed for Drp1-independent fission? When an understanding of molecular mechanism is combined with physiological insight, the study of mitochondrial dynamics will likely have a broad impact on our ability to modulate human health.

SUMMARY POINTS

1. Mitochondria are dynamic organelles that continually undergo fusion and fission. These opposing processes control the morphology of mitochondria and, more importantly, regulate their function.
2. Mitochondrial fusion is a multistep process. Outer membrane fusion depends on the mitofusins Mfn1 and Mfn2, which are transmembrane GTPases localized to the mitochondrial outer membrane. Inner membrane fusion depends on the dynamin-related GTPase OPA1.
3. Mitochondrial fission requires recruitment of the dynamin-related GTPase Drp1 from the cytosol onto the mitochondrial surface. Candidate receptors for Drp1 include Fis1, Mff, MiD49, and MiD51.
4. Mitochondrial dynamics is coordinated with cell physiology. Mechanisms include phosphorylation, proteolytic processing, and proteolytic degradation of proteins involved in mitochondrial dynamics.
5. Mitochondrial fusion and fission result in content exchange between mitochondria. These exchange events keep the mitochondrial population homogeneous and functional.
6. Mitochondrial dynamics is involved in multiple mitochondrial functions, including mtDNA stability, respiratory function, apoptosis, response to cellular stress, and mitochondrial degradation.
7. Several human diseases directly involve proteins critical for mitochondrial fusion or fission. In terms of fusion, heterozygous mutations in *OPA1* cause dominant optic atrophy, and heterozygous mutations in *Mfn2* cause the peripheral neuropathy Charcot-Marie-Tooth type 2A. In terms of fission, a mutation in *Drp1* has been linked to neonatal lethality with multisystem failure.

FUTURE ISSUES

1. Many mechanistic issues concerning mitochondrial fusion remain to be resolved. After tethering of the mitochondrial outer membranes, it is unclear how membrane merger is accomplished. For inner membrane fusion, it is similarly unclear how membrane deformation and tubulation by OPA1 results in membrane merger.
2. The mechanism of Drp1 recruitment to mitochondria remains unresolved. Currently, the best-characterized Drp1 receptor is Mff, but the roles of Fis1, MiD49, and MiD51 are poorly understood.
3. Most studies on mitochondrial dynamics have relied on cultured cells. To understand the role of mitochondrial dynamics in tissues, better animal models and imaging technologies are needed.
4. Although mitochondrial dynamics is a ubiquitous process, it is subject to regulatory mechanisms. Our current knowledge of regulatory mechanisms is superficial, and it will be important to integrate regulatory mechanisms with cell physiology.

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