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Mitochondrial Dynamics and Its Involvement in Disease

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Abstract

The dynamic properties of mitochondria—including their fusion, fission, and degradation—are critical for their optimal function in energy generation. The interplay of fusion and fission confers widespread benefits on mitochondria, including efficient transport, increased homogenization of the mitochondrial population, and efficient oxidative phosphorylation. These benefits arise through control of morphology, content exchange, equitable inheritance of mitochondria, maintenance of high-quality mitochondrial DNA, and segregation of damaged mitochondria for degradation. The key components of the machinery mediating mitochondrial fusion and fission belong to the dynamin family of GTPases that utilize GTP hydrolysis to drive mechanical work on biological membranes. Defects in this machinery cause a range of diseases that especially affect the nervous system. In addition, several common diseases, including neurodegenerative diseases and cancer, strongly affect mitochondrial dynamics.

INTRODUCTION

Mitochondrial DNA (mtDNA): a circular, 16.5-kb genome, located within the mitochondrial matrix, that encodes for 37 genes

Oxidative phosphorylation (OXPHOS): a metabolic, mitochondrial pathway, involving Complexes I to V, that efficiently generates ATP by oxidizing compounds via redox reactions

In the last decade, there has been an explosion in knowledge about the role of mitochondrial dysfunction in human pathologies. Although best known for their central function in energy production, mitochondria also have important roles in regulating apoptosis, calcium handling, innate immunity, and phospholipid synthesis. These diverse functions make mitochondria important for all cell types, but clinical evidence suggests that energy-intensive cells are particularly vulnerable to mitochondrial dysfunction. Diseases caused by primary mitochondrial dysfunction typically show prominent defects in skeletal muscle, cardiac muscle, and neurons.

Mitochondria are thought to be descendants of an ancient prokaryote that underwent an endosymbiotic event with an early eukaryote (1). Evidence of this evolutionary origin comes from phylogenetic analysis of mitochondrial genes, including the ribosomal RNAs, which show homology to those of Alphaproteobacteria. After more than one billion years of evolution, most of the original prokaryotic genome appears to have colonized the host genome, leaving the current mammalian mitochondrial DNA (mtDNA) genome with only 13 protein-encoding genes. Each of these 13 polypeptides is an essential component of the oxidative phosphorylation (OXPHOS) machinery that generates ATP aerobically and is much more efficient at energy extraction than anaerobic glycolysis alone. To translate these polypeptides, the mtDNA additionally encodes for 2 ribosomal RNAs that are components of the mitoribosomes and 22 transfer RNAs that are used for translation. All 37 genes of the mitochondrial genome, therefore, are important for OXPHOS activity, and high levels of mtDNA mutations generally result in impaired energy generation (2). However, the vast majority of the ~1,000 proteins of the mitochondrial proteome are encoded in the nuclear genome, are synthesized in the cytosol, and must be imported into the organelle.

Mitochondria are remarkably dynamic organelles. A fibroblast can have a population of dozens to hundreds of mitochondria, each containing at least one mtDNA genome. Individual mitochondria can have a variety of morphologies, appearing as small spheres, short or long tubules, or interconnected tubules. These morphologies are controlled by the opposing processes of fusion and fission. For example, short tubules can fuse together to form long tubules, and tubules can divide by fission into small spheres. Another dynamic aspect of mitochondria is the selective removal of dysfunctional mitochondria, a quality-control mechanism that ensures a healthy mitochondrial population. In many cells, mitochondria are highly motile and move through the cytosol via transport along the cytoskeleton. The term mitochondrial dynamics encompasses the processes of fusion, fission, selective degradation, and transport. This review summarizes recent developments in our understanding of the first three of these processes and their roles in human pathology. The focus is on mammalian mitochondrial biology, but insights from simpler model organisms are noted. Mitochondrial transport has been reviewed extensively elsewhere.

MITOCHONDRIAL FUSION

Characteristics of Fusion

Mitochondrial fusion is the merger of two mitochondria into one. In a typical mitochondrial fusion reaction, two mitochondria collide end-to-end, and the membrane fusion event occurs at the site of collision. Fusion reactions can also occur end-to-side, or within a single mitochondrion to form ringlike structures. Because mitochondria have double membranes, fusion consists of outer membrane fusion followed by inner membrane fusion. These two membrane fusion events occur close in time, but occasionally the temporal separation between these two events can be distinguished (3). The end result of membrane fusion is content mixing, with the matrix components diffusing throughout the new mitochondrion. Some fusion events are extremely brief, and the

KISS-AND-RUN FUSION EVENTS

In addition to obvious fusion events, where two mitochondria clearly merge into a larger mitochondrion, mitochondrial fusion can also take place via transient, so-called kiss-and-run encounters, in which no obvious merger or structural rearrangement occurs (3). Cells expressing photoactivatable fluorescent proteins targeted to the mitochondrial matrix are used to observe such events. When individual mitochondria are labeled by photoactivation with a laser, the labeled mitochondria often exchange matrix contents with other mitochondria during transient encounters that do not result in morphological merging. In cultured rat cardiac myocytes, these kiss-and-run fusion events constitute almost half of the fusion events that result in exchange of matrix proteins between mitochondria (3). These observations imply that fusion pores can rapidly open and close between two mitochondria. They further suggest that, apart from regulation of morphology, content exchange is an important function of mitochondrial fusion.

original mitochondria quickly separate after content exchange (see the sidebar titled Kiss-and-Run Fusion Events) (3). Although mtDNA is located in the matrix, mixing of mtDNA genomes after fusion appears limited (4).

Each cell type has a characteristic profile of mitochondrial morphology that is maintained by the balance between fusion and fission. For example, interphase fibroblast cells in culture typically have tubular mitochondria, ranging from short tubules to long tubules to interconnected tubular networks. When fusion is blocked, the tubules fragment rapidly into small spheres as a result of unopposed fission (5). When fission is blocked, the tubules elongate dramatically and show more interconnections due to unopposed fusion. Little is known about how cells maintain a particular balance between fusion and fission, but it has been observed that fission events often occur shortly after fusion events, suggesting feedback between the two processes (6).

Fusion Machinery

Mitochondrial fusion requires three large GTP-hydrolyzing enzymes of the dynamin superfamily. The mitofusins, Mfn1 and Mfn2, are located on the mitochondrial outer membrane and are required for outer membrane fusion. On the basis of observations that mitofusins must be present on opposing mitochondria for fusion to occur and that mitofusins form both homooligomeric and heterooligomeric complexes, it is commonly thought that *trans* interactions between mitofusins mediate tethering of mitochondria during the fusion process (7). Models of outer membrane tethering have been proposed (7–9) on the basis of structural studies. In the most recent models, guanine nucleotide–dependent dimerization of mitofusin mediates mitochondrial tethering (8, 9).

Inner membrane fusion is mediated by Optic Atrophy 1 (Opa1), also a member of the dynamin superfamily. Opa1 is associated with the inner membrane. Cells lacking Opa1 show mitochondrial outer membrane fusion, but such events never progress to inner membrane fusion (**Figure 1*a***) (10, 11). The abortive fusion intermediates resolve by fission, resulting in fragmented mitochondria in *Opa1*-null cells in spite of outer membrane fusion. Unlike mitofusins, Opa1 is needed on only one of two apposing mitochondria for inner membrane fusion to occur (10).

Dynamin: a large GTPase that hydrolyzes GTP to generate mechanical force to pinch off vesicles from the cell membrane during endocytosis

In human cells, eight RNA splice forms of Opa1 are generated from differential splicing of exons 4, 4b, and 5b (12). All RNA splice forms encode for a polypeptide containing an N-terminal mitochondrial targeting sequence (MTS) and the S1 proteolysis site encoded by the universal exon 5 (**Figure 1*b***). The MTS is removed by the matrix processing protease (MPP) during import of the N terminus into the mitochondrial matrix. If the S1 site is left intact, the resulting long isoform

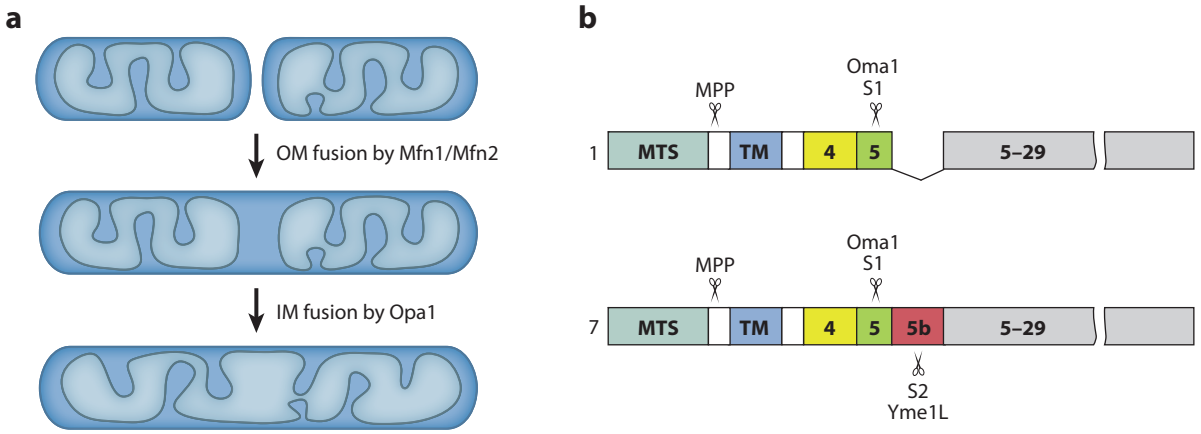

Figure 1

The fusion machinery. (*a*) Mitofusins mediate outer membrane (OM) fusion; Optic Atrophy 1 (Opa1) mediates inner membrane (IM) fusion. (*b*) Schematic of two of the eight RNA splice forms of Opa1. Isoform 1 generates a precursor whose N-terminal mitochondrial targeting sequence (MTS) is cleaved upon import to yield long Opa1. This long form can be further processed by Oma1 at the S1 site to yield short Opa1. For isoform 7, the long form can be processed by either Oma1 at the S1 site or Yme1L at the S2 site. Abbreviations: MPP, matrix processing protease; TM, transmembrane.

of Opa1 is anchored to the inner membrane through a transmembrane segment, with most of the protein facing the intermembrane space. If the S1 site is cleaved by the Oma1 protease, a short isoform of Opa1 is produced. These short isoforms do not have a membrane anchor and could in principle be soluble, but they form complexes with the membrane-bound long Opa1 isoforms to modulate fusion activity. Four splice forms (4, 6, 7, and 8) additionally contain the S2 proteolysis site (encoded by the differentially spliced exon 5b), which can be cleaved by the Yme1L protease to produce variant short isoforms. The Oma1 and Yme1L proteases therefore regulate Opa1 processing and modulate fusion activity in response to cellular conditions.

The differential splicing of exon 5b provides additional diversity in the short forms that are produced. An intriguing question is why this diversity of proteolytic processing of Opa1 exists. This is a controversial issue due to the difficulty of reconciling several diverging observations concerning the requirements of Opa1 to fuse mitochondrial membranes. It is clear that, under basal conditions, a combination of both long and short isoforms is required for physiological levels of mitochondrial fusion (13). For example, when *Opa1*-null cells are reconstituted with a complementary DNA that expresses only long Opa1 or only short Opa1, little mitochondrial fusion is detected. When both isoforms are coexpressed, substantial levels of mitochondrial fusion result (13). Under pathological conditions, such as dissipation of mitochondrial inner membrane potential, Oma1 is activated and promotes complete processing of Opa1 to short isoforms (14). This complete shift to short isoforms results in inactivation of fusion activity. It has also been proposed that short Opa1 promotes fission (15).

Nevertheless, several lines of evidence indicate that long Opa1 by itself is capable of mediating mitochondrial fusion. In *Opa1*-null cells expressing a noncleavable version of long Opa1, there is extensive elongation of mitochondria if the cells are treated with cycloheximide, which induces a condition called stress-induced hyperfusion (16). Additional evidence for the fusogenic activity of long Opa1 comes from the presence of mitochondrial fusion in cells lacking both the Oma1 and Yme1 proteases. These cells cannot process Opa1 into the short form, yet they contain some tubular mitochondria and mitochondrial fusion activity (15). Finally, in an in vitro assay, recombinant

long Opa1 incorporated into liposome membranes showed fusion activity against other liposomes, as long as the apposing liposomes contained cardiolipin (17). However, there is also evidence that short Opa1 can modulate or mediate fusion. The in vitro membrane fusion activity of long Opa1 is enhanced by addition of short Opa1 (17). Even more remarkably, short Opa1 alone mediates GTP-dependent fusion when added to liposomes containing cardiolipin (18).

Taken together, these results indicate that both the long and short forms of Opa1 can display inherent fusion activity. In cells under basal conditions, long Opa1 alone or short Opa1 alone shows little or no fusion activity (13). Under stress conditions, especially conditions associated with stress-induced hyperfusion (16), the fusion activity of the long isoform is activated by unknown mechanisms. Short Opa1 can substantially regulate the fusion activity of long Opa1 (13, 17), and in fact, cleavage of long Opa1 into the short isoform activates its membrane fusion activity (10).

In addition to fusion of the inner membrane, Opa1 plays an independent role in maintaining cristae structure. In the absence of Opa1, the ultrastructure of the cristae is severely disrupted, and the respiratory chain supercomplexes are greatly reduced (19). The role of Opa1 in cristae structure is independent of its fusion activity. In *Opa1*-null cells, expression of RNA isoforms that do not show mitochondrial fusion activity nevertheless rescues cristae structure (20, 21).

Cardiolipin: a dimeric phospholipid that is highly enriched in the mitochondrial inner membrane

Cristae: mitochondrial inner membrane infoldings that are enriched for enzymes involved in oxidative phosphorylation

Respiratory chain supercomplexes: higher-order assemblies of respiratory chain complexes located in the mitochondrial inner membrane; an example is the 1.7-MDa $I_1III_2IV_1$

MITOCHONDRIAL FISSION

The Central Role of Drp1

Mitochondrial fission is the division of a mitochondrion into two smaller mitochondria. The central mediator of mitochondrial fission is dynamin-related protein 1 (Drp1), a GTP-hydrolyzing enzyme (22). This protein is recruited from a cytosolic pool onto the mitochondrial surface, where it self-assembles into spiral structures that wrap around and constrict mitochondrial tubules to facilitate fission. In this manner, it functions analogously to classical dynamins in constriction of the necks of endocytic invaginations of the cell membrane (23). In addition to its role in mitochondrial fission, Drp1 and several other components of the fission machinery localize to peroxisomes, and loss of Drp1 causes elongation of peroxisomes.

The biophysical properties of these dynamin superfamily proteins are tailored to the structures (mitochondrial tubule versus endocytic invagination) that they constrict. It has been suggested that tubule constriction by Drp1 alone is inadequate to fully mediate tubule scission, due to its inability to constrict the tubules sufficiently (24). The classical dynamin Dnm2 was also found to be recruited to mitochondria at sites of fission. Dnm2 knockdown cells are notable for mitochondria with very narrow constrictions that were proposed to represent Drp1-generated fission intermediates that fail to undergo scission (24). However, the model that Dnm2 is a core part of the fission machinery that acts after Drp1 to catalyze membrane scission must be further evaluated. In contrast to knockdown, cells lacking either Dnm2 or all three classical dynamins (Dnm1, Dnm2, and Dnm3), generated by CRISPR (clustered regularly interspaced short palindromic repeats) gene editing, show no evidence for deficiency in either mitochondrial or peroxisomal fission (25).

Recruitment of Drp1 to Fission Sites

Recruitment of Drp1 from the cytosol onto the mitochondrial surface requires the action of Drp1 receptors residing on the mitochondrial outer membrane. This concept is best illustrated in yeast, where the outer membrane protein Fis1 recruits Dnm1 (yeast ortholog of Drp1) via the molecular adaptors Mdv1 and Caf4 (26). In the absence of Fis1 or the adaptors, yeast Dnm1 remains cytosolic. Mammals also contain Fis1, but its role in Drp1 recruitment and mitochondrial fission appears

minor. Mammalian Fis1 clearly does not play the central role in mitochondrial fission that yeast Fis1 does—cells lacking Fis1 show little or no fission deficiency (27, 28). Instead, Fis1 is required for some forms of mitophagy (29–31). Whether this requirement for Fis1 reflects a function in a specialized form of fission, or whether Fis1 coordinates the association of mitochondria with autophagosomal membranes, remains unclear (29).

Three other outer membrane proteins—Mff, MiD49, and MiD51—have more substantial roles in recruitment of Drp1 to mitochondria. Depletion of any of these molecules results in notable elongation of mitochondria, and depletion of all three causes a fission defect similar in severity to Drp1 depletion (28, 32, 33). The function of Mff seems straightforward in the sense that deletion reduces Drp1 recruitment to mitochondria and impairs fission, whereas overexpression of Mff enhances mitochondrial fragmentation. In contrast, the mode of MiD action is more complicated. Low-level expression results in enhanced mitochondrial fission, but overexpression causes dramatic elongation of mitochondria. Although each protein can independently recruit Drp1 to mitochondria (28, 34), there are indications that MiDs and Mff physically interact with one another in a Drp1 complex to modulate fission (33, 35). The details of such interactions and how they regulate fission remain to be clarified.

After its recruitment to mitochondria, Drp1 appears to undergo substantial structural changes to constrict the mitochondrial tubule and induce the scission event. A puzzling aspect of MiD49 and MiD51 biology is that overexpression of these proteins leads to extensive recruitment of Drp1 to mitochondria but paradoxically causes mitochondrial elongation. These elongated mitochondria can be triggered to undergo rapid fission when cells encounter stress, leading to the proposal that the MiDs recruit an inactive conformation of Drp1 that can subsequently be activated (36). Recombinant Drp1 and MiD49 form a stable copolymer only when Drp1 is bound to GMPPCP, a nonhydrolyzable form of GTP. High-resolution cryogenic electron microscopy (cryo-EM) analysis indicates that this copolymer is a linear filament in which extensive Drp1–MiD49 packing at four distinct interfaces likely stabilizes Drp1 in a GTP conformation that seems incompatible with scission (37). Remarkably, exchange of the GMPPCP for GTP and subsequent GTP hydrolysis convert the linear filaments into rings with an internal diameter of 16 nm. In this ring conformation of Drp1, MiD49 is dissociated. These observations lead to a model in which GTP-bound Drp1 is recruited by MiD49 and assembles into linear filaments that must undergo extensive conformational changes to mediate fission (37). Hydrolysis of the GTP leads to MiD49 dissociation, followed by rearrangement of the Drp1 filament into a ring that constricts the mitochondrial tubule. Additional cryo-EM studies indicate that cardiolipin, a lipid enriched in mitochondrial membranes, can modulate Drp1 structure and enhance its GTP-hydrolyzing activity (38).

Interorganellar Interactions

Physical interactions between the endoplasmic reticulum (ER) and mitochondria appear to play a role in determining the sites of mitochondrial fission. Contact sites between mitochondria and ER have long been studied with electron microscopy and are important for coordination of phospholipid synthesis and calcium signaling between the two organelles. Biochemical studies also indicate that a fraction of ER membranes, termed mitochondria-associated membranes, copurify with mitochondria. Live-cell imaging studies indicate that, in both yeast and mammalian cells, the majority of mitochondrial fission sites show ER tubules crossing or wrapping around the mitochondria (39). Mff, MiD49, MiD51, and Drp1 often colocalize with these ER-marked sites, where the mitochondrial tubules are constricted (39, 40). Interestingly, these ER-marked constrictions persist in the absence of Mff or Drp1, suggesting that ER wrapping may be an initial step in the fission process, perhaps reducing the diameter of the tubule down to the dimensions of Drp1 spirals.

Sites of mitochondrial division also appear to be influenced by the dynamics of mtDNA. mtDNA is associated with numerous proteins that regulate its structural organization or metabolism, including single-stranded DNA–binding protein, transcription factor A, and Twinkle helicase (41). The mtDNA is compacted into nucleoid structures that show dynamic behavior within mitochondria, including division events to separate replication products (42). In wild-type cells, most mitochondrial fission events occur close to nucleoids (43). Cells lacking Drp1 have abnormally large mtDNA nucleoids, which are caused by clustering of nucleoids (43, 44). These enlarged nucleoids cause regional bulging of mitochondrial tubules in Drp1 knockout cells. In cardiomyocytes, these defects in nucleoid morphology lead to reduced respiratory chain function (44). Only a fraction of ER–mitochondria contacts ultimately result in mitochondrial fission. The positions of nucleoids that are actively replicating are highly correlated with the subset of contacts that ultimately progress to fission (45). This observation suggests that mtDNA replication provides a signal to induce mitochondrial fission.

BENEFITS OF MITOCHONDRIAL FUSION AND FISSION

Knockout experiments clearly indicate that fusion and fission are important for mitochondrial function. Inhibiting either process in cells or animals results in severe mitochondrial dysfunction. Several cellular mechanisms underlie these beneficial effects (**Figure 2**).

Regulation of Mitochondrial Morphology

The net balance between mitochondrial fusion and fission controls the size, number, and shape of mitochondria (**Figure 2*a***). For example, an increase in fission or reduction in fusion will result in smaller, more numerous mitochondria in the form of short tubules or spheres. In some cells, the shape per se has important functional consequences. In neurons, the transport of small mitochondria to nerve terminals may be more efficient than that of long mitochondria (**Figure 2*b***). When mitochondrial fission is blocked in neurons, the elongated mitochondria are less efficient at entering neuronal extensions to support synapses; conversely, overexpression of Drp1 increases dendritic mitochondria and is associated with enhanced density of synapses (46, 47). The failure of elongated mitochondria to be transported into nerve terminals may result from entanglement of long mitochondria in the soma. However, note that small mitochondria do not always show efficient transport. Mitochondria that are fragmented due to impaired fusion have severe transport defects (5). There is evidence that the mitofusins physically interact with Miro and Milton, which form an outer membrane protein complex involved in the transport of mitochondria along microtubules (48).

Mitochondrial shape may directly affect the bioenergetic function of mitochondria, with elongated mitochondria sometimes correlated with more efficient ATP production. However, the causal connection between mitochondrial shape and bioenergetics remains unclear. There is no question that many types of mitochondrial dysfunction, whether caused by administration of an uncoupler or resulting from pathology, result in fragmentation of mitochondria. In some cases, mitochondrial dysfunction activates the Oma1 protease, leading to excessive processing of Opa1 at the S1 cleavage site (49). The conversion of the Opa1 pool into exclusively short isoforms impairs mitochondrial fusion. In addition, energy deficiency causes activation of AMP-activated protein kinase, which phosphorylates Mff to promote mitochondrial fission (50). Although mitochondrial dysfunction will often cause mitochondrial fragmentation, it does not mean that fragmented mitochondria should generally be equated to low ATP production without experimental confirmation. Short isoforms of Opa1 are deficient in mitochondrial fusion but will rescue ATP production in *Opa1*-null cells, even though the mitochondria remain fragmented (20, 21).

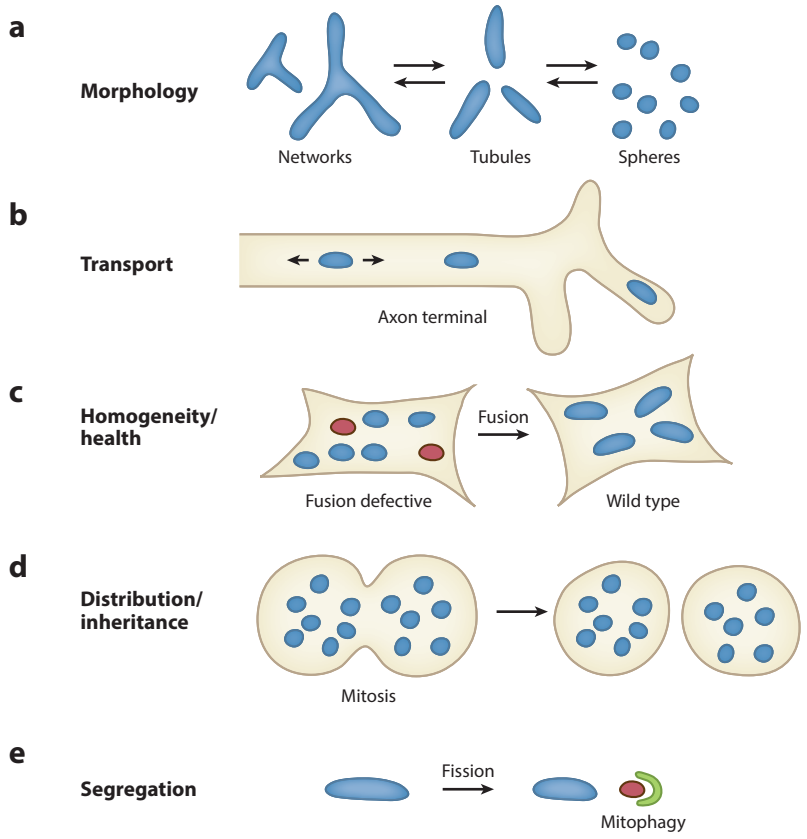

Figure 2

The benefits of mitochondrial dynamics. Schematic showing how mitochondrial fusion and fission affect mitochondrial function. Red indicates a damaged or dysfunctional mitochondrion. (*a*) The balance between fusion and fission controls mitochondrial shape, number, and size. (*b*) Mitochondrial fission results in smaller mitochondria that are more efficiently transported in axons. (*c*) Mitochondrial fusion promotes maintenance of a homogeneous mitochondrial population that can tolerate higher levels of mitochondrial DNA mutations. (*d*) Mitochondrial fission during mitosis facilitates equitable inheritance by daughter cells. (*e*) Mitochondrial fission generates small mitochondrial fragments that can be engulfed by autophagosomes during mitophagy.

Promoting Content Exchange

A key function of mitochondrial fusion and fission is to promote mixing of membranes and contents between mitochondria. An important role for content exchange can be inferred from the extensive occurrence of kiss-and-run fusion events (see the sidebar), which lead to content exchange without affecting mitochondrial morphology (3). Content exchange has several benefits for mitochondria. First, it promotes homogenization of the mitochondrial population (**Figure 2*c***). Because the mtDNA encodes for only 13 polypeptides, mitochondria import most of their proteome from the cytosol. When mitochondrial fusion is inhibited, individual mitochondria diverge in their properties, as measured by immunostaining against key mitochondrial markers (51, 52). This observation indicates that fusion helps to reduce organelle-to-organelle variability. Second, mitochondrial fusion helps ameliorate the detrimental effects of heteroplasmic mtDNA mutations. In mitochondrial diseases caused by mtDNA mutations, the mutations are typically

recessive, and the mutational load has to reach high levels, approximately 60–90% heteroplasmy, before cells encounter respiratory chain dysfunction. This ability to tolerate high levels of mtDNA mutations is dependent on mitochondrial fusion (51).

Maintenance of Mitochondrial DNA

Both mitochondrial fusion and fission are necessary to maintain mtDNA. In the absence of mitochondrial fusion, cells show a drastic reduction in the amount of mtDNA. The mtDNA defect is complete in yeast cells but partial in mammalian cells (51). In addition, the distribution of mtDNA nucleoids in mutant mammalian cells is defective, such that many mitochondria lack any evidence of mtDNA and therefore are unable to maintain OXPHOS activity (53). In many cell types, severe loss of mitochondrial fusion causes reduced OXPHOS activity. It is unknown whether the OXPHOS defect is due entirely to the reduced mtDNA levels or whether additional mechanisms related to fusion are involved. Opa1 is known to have a role in cristae structure and OXPHOS supercomplex maintenance that is independent of its role in inner membrane fusion (19–21).

In the absence of mitochondrial fission, mtDNA nucleoids aggregate, forming large structures that deform mitochondrial tubules (43). The clustering of nucleoids results in uneven distribution of mtDNA within the mitochondria and causes mosaic OXPHOS deficits in cardiomyocytes (44). OXPHOS activity is concentrated around the clustered nucleoids and absent elsewhere. In contrast, fibroblasts lacking Drp1 do not show OXPHOS defects (54).

Inheritance of Mitochondria

During the cell cycle, mitochondrial morphology undergoes marked transitions (55) that likely facilitate even distribution of mitochondria to daughter cells (**Figure 2*d***). In tissue culture cells, mitochondria are elongated at the G_1–S transition but fragment at the G_2 and M phases (55). This fragmentation is promoted by mitotic phosphorylation of Drp1 that enhances its fission activity and results in many more mitochondria during mitosis (56, 57). Because the mitochondria are associated with cytoskeletal elements distributed throughout the cell, this fragmentation may help promote equitable partitioning of mitochondria to the two daughter cells. In cultured mammalian cells, it has been assumed that mitochondrial inheritance is passive and occurs secondary to the even partitioning of cytosolic contents to daughter cells. However, there is evidence that mammary stemlike cells transmit mitochondria asymmetrically, such that progeny maintained as stem cells receive fewer aged mitochondria (58). This phenomenon suggests that active mechanisms for mitochondrial inheritance do exist in some mammalian cell types. In budding yeast, there are well-defined mechanisms for actively transporting mitochondria into the developing bud, as well as for maintaining another set of mitochondria in the mother cell (59).

Segregation of Damaged Mitochondria

Mitophagy, the degradation of mitochondria by autophagy, is a major mechanism for mitochondrial quality control. Dysfunctional mitochondria are recognized by the autophagic machinery, resulting in their engulfment by autophagosomes and trafficking to the lysosome for degradation. There are multiple mitophagy pathways, including one mediated by Pink1 and Parkin, molecules involved in familial forms of Parkinson's disease (detailed in the section titled Parkinson's Disease) (60). In order for dysfunctional mitochondria to be culled, they must be segregated from the rest of the mitochondrial population. This segregation arises in several complementary ways. First, the dysfunctional mitochondria are prevented from fusing with other mitochondria. Mitochondrial dysfunction that involves inner membrane depolarization results in Oma1 activation and induced

Autophagy: a set of processes that degrade or recycle cellular components by delivery to the lysosome

Autophagosome: a double-membraned vesicle that segregates cellular contents and delivers them to the lysosome for degradation

Inner membrane depolarization: loss of the membrane potential across the mitochondrial inner membrane

processing and inactivation of Opa1 (6, 14, 49). In addition, Opa1 function is responsive to OXPHOS activity of mitochondria, irrespective of membrane uncoupling. Mitochondria that do not actively respire are unable to fuse (10). Second, mitochondrial fission promotes segregation of mitochondria and may also be important for generating mitochondrial fragments of the appropriate size for engulfment by autophagosomes (**Figure 2*e***). In yeast, the mitophagy adaptor Atg32 forms a complex with Atg11, which connects mitochondria with autophagosomes. Atg11 also recruits Dnm1, thereby directly coordinating fission of mitochondria and their engagement with autophagosomes (61). In mammalian cells, inhibition of Drp1 reduces Parkin-mediated mitophagy (62). However, some forms of mitophagy do not require the action of Drp1. In such cases, the isolation membrane wraps around a portion of a mitochondrion, and the closure of the isolation membrane into an autophagosome seems to pinch off a piece of the organelle (63). Finally, in Parkin-mediated mitophagy, many outer membrane proteins are degraded by the ubiquitin–proteasome system, including the mitofusins and Miro (64, 65). Removal of these proteins prevents mitochondrial fusion and transport.

Ubiquitin: a protein (76 residues in humans) that is covalently conjugated onto lysine residues of substrate proteins to regulate their function

Whereas fission promotes segregation of damaged mitochondria, there is evidence that fusion can interfere with this process. In a fly model containing heteroplasmic mtDNA deletions, reduction of mitofusin levels leads to lower levels of the mutant mtDNA (66). This result suggests that mitochondrial fusion reduces the efficiency of culling defective mtDNA, likely by reincorporating defective organelles into the mitochondrial population.

Balancing Fusion and Fission

Knockout studies clearly indicate that unilateral loss of fusion or fission has severe detrimental effects on mitochondrial function. However, the proper balancing of mitochondrial fusion and fission appears to be more important than the absolute level of each process. Mouse lacking *Mff* show a severe reduction in mitochondrial fission and die at 12–14 weeks from dilated cardiomyopathy. Cardiomyocytes show greatly reduced OXPHOS activity, and the cardiac tissue has extensive fibrosis. These defects are completely rescued by simultaneous loss of *Mfn1* (67). Moreover, animals lacking *Mfn1* normally show neonatal lethality, which is, in turn, rescued by the simultaneous loss of *Mff*. Therefore, the combination of two lethal mitochondrial dynamics mutations results in a largely normal mouse. This finding indicates that low levels of mitochondrial fusion and fission are compatible with high cellular and tissue function, as long as the two processes are appropriately balanced. It remains possible that these double-mutant mice are sensitive to certain types of stress, but robust mitochondrial function is apparently restored by rebalancing of mitochondrial dynamics. These results suggest that rebalancing of mitochondrial dynamics may provide a therapeutic strategy for diseases associated with defects in mitochondrial fusion or fission. In fact, there is evidence for cellular feedback mechanisms that work to partially rebalance mitochondrial dynamics when fission is perturbed. For example, when *Drp1* is deleted, the levels of Mfn1 and Mfn2 are reduced by ~50% (54, 68). This compensatory response presumably results in partial rebalancing of mitochondrial dynamics, though it is insufficient to prevent dysfunction in some cell types.

MITOCHONDRIAL FUSION AND DISEASE

Charcot–Marie–Tooth Disease Type 2A

Heterozygous mutations in *MFN2* cause Charcot–Marie–Tooth disease (CMT) type 2A (CMT2A), an autosomal dominant peripheral neuropathy characterized by sensory and motor deficits in the distal limbs (**Table 1**) (69, 70). Motor defects are more prominent than sensory

Table 1 Diseases caused by primary defects in mitochondrial fusion or fission

Gene	Disease	Inheritance	Typical features
MFN2	CMT2A	Autosomal dominant	Distance-dependent, peripheral sensorimotor neuropathy, more severe in lower limbs (70). Less common features are optic atrophy (when present, sometimes designated as HMSN VI), encephalopathy (94), mtDNA deletions in skeletal muscle (96)
	HMSN V	Autosomal dominant	Peripheral neuropathy with pyramidal signs (such as extensor plantar response and some increase in muscle tone in lower extremity) (95)
	MSL	Recessive	Peripheral neuropathy, upper body adipose overgrowth (89)
	Canine fetal-onset neuroaxonal dystrophy	Recessive	Death at birth due to pulmonary hypoplasia, cerebellar and spinal cord underdevelopment, fixed joints (86)
OPA1	DOA	Autosomal dominant	Vision loss due to degeneration of optic nerve (153)
	DOA-plus	Autosomal dominant	Vision loss, peripheral neuropathy, deafness, ataxia, progressive external ophthalmoplegia, myopathy with mtDNA deletions (92, 93)
MFF	Rare cases of mitochondrial disease	Recessive	Delayed childhood development, optic atrophy, seizures, peripheral neuropathy, hypotonia, abnormalities in brain MRI (106, 107)
DRP1	Single case of neonatal lethality	Heterozygous, de novo mutation	Multisystem failure, including microcephaly, optic atrophy, hypoplasia, lactic acidemia (101)
	Intractable forms of epilepsy	Mostly heterozygous, de novo mutations	Intractable epilepsy, developmental delay (102–104)
SCL25A46	Optic atrophy spectrum disorder	Recessive	Optic atrophy, peripheral neuropathy, cerebellar atrophy (111)
	Leigh syndrome	Recessive	Developmental delay, lesions in brain stem and midbrain (112)
	Congenital pontocerebellar hypoplasia	Recessive	Neonatal lethality, small cerebellum and brain stem (113)
	Progressive myoclonic ataxia	Recessive	Ataxia, myoclonus, optic atrophy, cerebellar atrophy (115)

Abbreviations: CMT2A, Charcot–Marie–Tooth disease type 2A; DOA, dominant optic atrophy; HMSN, hereditary motor and sensory neuropathy; MRI, magnetic resonance imaging; MSL, multiple symmetric lipomatosis; mtDNA, mitochondrial DNA.

ones, and common features include a gait defect, weakness in the feet and hands, and hypotonia. Clinical features indicate that the neuropathy is distance dependent, because distal regions of limbs are most severely affected and the proximal regions are largely spared. Unlike demyelinating forms of CMT, CMT2A is an axonopathy, and there is typically no change or only a slight decrease in nerve conduction velocity. Most cases are caused by heterozygous mutations in *MFN2* and show onset by 20 years of age, but some late-onset cases have been reported. Some unusual cases with homozygous or compound heterozygous mutations have also been reported (71); these alleles are likely to be only partial loss-of-function mutations, since severe alleles would be expected to be lethal when homozygous.

Axonopathy: a disorder affecting primarily the axons of peripheral nerves, as opposed to primarily myelin

It has been challenging to develop robust animal models for CMT2A. Mice containing a knock-in of the *R94W* mutation, which causes early-onset disease in humans, are almost normal and show only a mild reduction in activity in an open field test (72). They lack classical features of CMT, such as distal limb neuropathy or gait defects. Mice homozygous for the *R94W* mutation die shortly after birth (72), consistent with the observation that this mutation abolishes mitochondrial fusion (73).

Most *MFN2* mutations associated with CMT2A cause mitochondrial aggregation when overexpressed in cultured cells (73), suggesting that they have at least a partial dominant mode of action. Several transgenic mouse models confirm this view. Overexpression of the *T105M* (74, 75) or *R94W* mutation (76) in transgenic mice results in a variety of neurological phenotypes, including locomotor impairment. These phenotypes are associated with clumping of mitochondria in neurons and sparsity of mitochondria in axons due to poor trafficking. Consistent with this phenotype, cultured fibroblasts and neurons lacking mitofusins have impaired transport (5, 48), and both mitofusins physically interact with the Milton–Miro complex, at least when overexpressed (48). These mouse models highlight the dominantly acting nature of some CMT2A alleles, although more research is needed to confirm that these effects reflect the molecular pathology in patients with CMT2A. A transgenic fly model suggests that the common *R364W* CMT2A allele is hyperactive for mitochondrial fusion and can be rescued by overexpression of Drp1 (77). Expression of *R364W* causes mitochondrial damage and the appearance of autophagic bodies.

In addition to mitochondrial defects, CMT2A may involve impairment of ER–mitochondria contacts (78). The ER has intimate contacts with mitochondria that are important for regulating phospholipid biosynthesis, calcium signaling, and mitochondrial fission. A subset of Mfn2, though not Mfn1, localizes to the ER, where it is proposed to play a role in ER–mitochondria tethering (78). However, whether Mfn2 promotes or inhibits ER–mitochondria tethering is disputed, with conflicting data from several groups (79–81). ER–mitochondria contacts have been examined in only a handful of CMT2A patient cells, and the data have not been definitive. Mild defects were reported in two cell lines, and no defects were found in two others (82, 83). A transgenic mouse model overexpressing $Mfn2^{R94W}$ was found to have reduced ER–mitochondria contacts (82), but as mentioned above, the physiological significance of this model of overexpression needs to be confirmed.

The importance of mitofusins in neuronal function has been clearly demonstrated in mouse models. Deletion of *Mfn2* results in embryonic lethality, but conditional knockouts have indicated a critical function for Mfn2 in a variety of neurons, including Purkinje cells of the cerebellum and dopaminergic neurons (53, 84, 85). On the basis of these mouse phenotypes, one might predict that severe, homozygous loss-of-function mutations of *MFN2* in humans are likely to result in phenotypes much more severe than those found in CMT2A and are incompatible with viability. Consistent with this view, an inherited, fatal neuroaxonal dystrophy in a large family of dogs has been traced to a homozygous *MFN2* mutation that removes a glutamine residue and almost entirely eliminates steady-state levels of protein (86). This disorder causes peripheral and central nervous system defects, including cerebellar hypoplasia with degeneration of Purkinje cells. Axonal swelling is found throughout the peripheral and central nervous systems. Pups die on the day of birth due to failure of the respiratory system. A clinically similar disorder, termed infantile neuroaxonal dystrophy, is found in humans, but thus far this disorder has not been associated with *MFN2*.

Multiple Symmetric Lipomatosis

A plethora of *MFN2* mutations, usually heterozygous, have been associated with CMT2A. In contrast, a single *MFN2* mutation, *R707W*, is uniquely associated with multiple symmetric lipomatosis

(MSL, also called Madelung's disease) when homozygous (87–89). This disease is highly variable but is characterized by symmetric growth of adipose tissue around the neck, shoulder, and proximal limbs. These lipomas are benign, but can be painful and disfiguring. In contrast to this deposition of fat in the upper body, the lower extremities show little adipose tissue. Although abnormal adipose deposition is the most striking aspect of this disease, there are additional metabolic and neurological features. Many patients have low leptin levels, hypertriglyceridemia, insulin resistance, and diabetes. CMT-like neuropathy is also present and can be the most debilitating aspect.

Because only the *R707W* mutation has been associated with MSL, it seems probable that this allele has a neomorphic function. Neither heterozygous mutation of *R707W* nor any other *MFN2* CMT2A mutation has been associated with MSL. Most of the MSL cases involving *MFN2* have homozygous *R707W* mutations, although a few have biallelic *MFN2* mutations, with one allele being *R707W*. Cellular studies indicate that $Mfn2^{R707W}$ has mildly reduced mitochondrial fusion activity (89). The mutation impairs self-interactions between Mfn2 molecules, but not between Mfn1 and Mfn2. Ultrastructural analysis of the adipose deposits shows expansion of the cytoplasmic rim of adipocytes. The mitochondria are enlarged and have abnormal cristae. Expression analysis indicates ER and mitochondrial stress and downregulation of OXPHOS proteins (88). These mitochondrial defects were specific to the adipose deposits and were not observed in patient fibroblasts. These observations indicate that the *R707W* mutation selectively leads to adipose hyperplasia and dysfunction. However, it remains to be determined whether this effect reflects a normal role of Mfn2 or a neomorphic function.

More broadly, there is substantial evidence for a role of mitochondrial dysfunction in MSL. Almost a third of patients with MSL have the m.8344A>G mtDNA mutation associated with MERRF (myoclonic epilepsy with ragged red fibers), mtDNA deletions, or ragged red fibers (a histological sign of mitochondrial dysfunction in skeletal muscle) (90). Also, 15–25% of patients with MERRF have multiple lipomatosis or lipomas. These observations indicate that a substantial fraction of MSL cases have an etiology related to mitochondrial dysfunction.

Lipoma: a benign tumor of adipose tissue

Neomorphic: refers to a type of mutation that confers a novel molecular function to a gene, unrelated to its normal function

Ragged red fiber: a pathological type of muscle fiber that is revealed by Gomori trichrome staining and is indicative of mitochondrial dysfunction

Retinal ganglion cell: a type of neuron in the retina with an axon transmitting visual information to the brain through the optic nerve

Dominant Optic Atrophy

Dominant optic atrophy (DOA) is the most common inherited optic neuropathy, and most cases are caused by heterozygous mutations in *OPA1* (91). As discussed above, Opa1 is essential for mitochondrial inner membrane fusion and also plays a prominent role in maintaining cristae membrane ultrastructure and OXPHOS supercomplexes. Many *OPA1* disease mutations have been identified, and at least some cases result from haploinsufficiency. Because Opa1 self-assembles into oligomers, dominant negative effects are likely involved in other cases. Classical DOA is characterized by progressive, bilateral loss of vision caused by degeneration of retinal ganglion cells, resulting in pallor of the optic nerve. However, some *OPA1* mutations are associated with the more severe DOA-plus syndrome, which has additional features such as hearing loss, myopathy, and peripheral neuropathy (92, 93). Pathologically, DOA-plus shows sporadic OXPHOS-defective muscle fibers that have mtDNA deletions, consistent with mouse studies showing that mtDNA maintenance and fidelity require mitochondrial fusion (51).

The classic forms of CMT2A and DOA, both caused by mutations in mitochondrial fusion genes, affect distinct cell types—motor and sensory neurons versus retinal ganglion cells, respectively. However, as more *MFN2* mutations are identified, it is becoming clear that there can be overlapping clinical features. Some *MFN2* mutations are associated with optic atrophy in addition to the classical peripheral neuropathy (69), thereby overlapping with DOA. Some particularly severe cases include encephalopathy (94), pyramidal signs (95), or mtDNA deletions in skeletal muscle (96), with the last finding resembling the so-called DOA-plus syndrome. As noted above, peripheral neuropathy, the key feature of CMT2A, is found in some patients with DOA-plus.

Adeno-associated virus (AAV): a virus of the Parvoviridae family that is a leading vector for in vivo delivery of genes

Because the cells of the retina are readily accessible through the vitreous cavity of the eye, there is interest in developing gene therapy for DOA by targeting the retinal ganglion cells for reexpression of *OPA1*. Such an approach would be especially promising for cases associated with *OPA1* haploinsufficiency, where simple restoration of Opa1 expression level would be expected to alleviate disease. Some of the technological groundwork for this approach has been developed for Leber's hereditary optic neuropathy (LHON) (97) and Leber's congenital amaurosis (98). LHON is a clinically similar blindness also caused by degeneration of retinal ganglion cells. LHON is maternally inherited, because it is caused by mtDNA mutations, most commonly in *MT-ND4*, a core component of NADH dehydrogenase. Adeno-associated virus (AAV) has been used to deliver an *ND4* gene reengineered to be expressed from the nuclear genome, with some encouraging results (97). In principle, a similar AAV approach can be used to deliver the wild-type *OPA1* gene to the retinal ganglion cells for DOA cases caused by haploinsufficiency. A proof-of-principle study using a mouse DOA model has shown promise in alleviating retinal ganglion cell degeneration (99).

In the context of gene therapy, one of the complications of *OPA1* is the diversity of RNA splice forms generated from the locus. As noted above, humans produce at least eight splice forms as a result of differential splicing of exons 4, 4b, and 5b (12). Each splice form encodes a polypeptide with one or more protease processing sites, resulting in the potential to generate long and short protein isoforms. Cellular studies indicate that physiological levels of mitochondrial fusion require a combination of long and short Opa1 isoforms. The diversity of RNA splice forms raises the issue of whether a single RNA form is capable of restoring all the functions of *OPA1* (100). When the various RNA splice forms are individually expressed in mouse *Opa1*-null cells, only isoforms that give rise to both long and short isoforms can complement the mitochondrial fusion defect (13). However, all the RNA splice forms are proficient at restoring mtDNA levels, cristae structure, and respiratory chain function (20, 21). How these different functions of Opa1 are related to the pathophysiology of DOA remains to be determined.

MITOCHONDRIAL FISSION AND DISEASE

Drp1

In humans, most disease-causing *DRP1* mutations appear to be de novo, heterozygous, and dominantly acting. The first reported mutation, *A395D*, resulted in neonatal lethality due to multisystem damage, including microcephaly, optic atrophy, and hypoplasia (101). Patient fibroblasts showed elongation of both mitochondria and peroxisomes. Subsequent cases of *DRP1* mutation have been less severe, with neurological symptoms predominating. In particular, the *R403C* mutation is associated with normal early childhood followed by refractory epilepsy that progresses to rapid neurological decline (102). The *R403C* mutation has now been found multiple times with similar clinical outcomes, and other *DRP1* mutations also cause epilepsy as a key feature (103, 104). Several *DRP1* mutations are expected to affect self-assembly and act in a dominant negative manner, and these features have been confirmed for *A395D* and *R403C* (102).

Deletion of *Drp1* in mice results in embryonic lethality and prominent neuropathology (54, 68). Genetic studies suggest multiple mechanisms that contribute to the role of mitochondrial fission in neurons. Disruption of *Drp1* in *Drosophila* neurons causes elongated mitochondria that congregate in the cell body and fail to traffic to the axon terminal (47). Mouse Purkinje neurons lacking Drp1 have large, spherical mitochondria with poor respiratory chain activity and markers of oxidative damage (105). These defects are improved by administration of antioxidants, suggesting that loss of mitochondrial fission in this cell type causes an increase in oxidative stress that impairs mitochondrial function (105).

Mff

Only four cases of *MFF* mutations causing disease have been documented. Each patient contained either homozygous or biallelic, truncating mutations (106, 107). In the three more severe cases, the clinical features arose within the first year and included microcephaly, hypotonia, epileptic seizures, spasticity, and optic neuropathy (107). The patients' fibroblasts showed extremely long and interconnected mitochondria and peroxisomes, and Drp1 was diffusely cytosolic instead of in punctate spots on mitochondria.

Retrotranslocation: the export of proteins from the lumen or membrane of an organelle back into the cytoplasm

SLC25A46

In yeast cells, Ugo1p is an outer membrane protein that is essential for mitochondrial fusion (108). Ugo1p belongs to the solute carrier family and physically interacts with both Fzo1p, the yeast mitofusin, and Mgm1p, the yeast Opa1 (109, 110). By associating with these two fusion machines, Ugo1p is thought to couple outer membrane fusion to inner membrane fusion. In humans, SLC25A46 belongs to the solute carrier family and has been proposed to be the ortholog of Ugo1p (111). Surprisingly, however, depletion of *SLC25A46* results in mitochondrial elongation (111–113). Despite this evidence for a profission role, SLC25A46 interacts with the fusion machinery (112, 114) and affects the stability of Mfn1 and Mfn2 (114). In addition, SLC25A46 interacts with MICOS (mitochondrial contact site and cristae organizing system) and EMC (ER membrane protein complex) subunits, leading to roles in cristae structure, ER structure, and mitochondrial phospholipid composition (112). Recessive mutations in *SLC25A46* cause a remarkable range of diseases, including optic atrophy with axonal CMT features (111), Leigh syndrome (112), pontocerebellar hypoplasia (113), and progressive myoclonic ataxia (115).

OTHER DISEASES AFFECTING MITOCHONDRIAL DYNAMICS

Parkinson's Disease

Two forms of inherited, early-onset Parkinson's disease are caused by mutations in the Pink1 serine/threonine kinase (PTEN-induced kinase 1, encoded by *PARK6*) and the E3 ubiquitin ligase Parkin (encoded by *PARK2*). The discussion in this section focuses on the role of the Pink1–Parkin pathway in mitochondrial degradation, although these proteins also have nonmitochondrial functions.

Genetic studies in flies indicate that Parkin works downstream of Pink1 to maintain mitochondrial function (116, 117). In mammalian cells, the Pink1–Parkin pathway constitutes a quality-control mechanism, termed mitophagy, that identifies and removes damaged mitochondria through autophagy (118). When mitochondria are healthy, very low levels of Pink1 are present in mitochondria due to high turnover (119). Pink1 is imported partially into the mitochondrial matrix through the presequence import pathway that involves translocase of the outer membrane (TOM) and translocase of the inner membrane (TIM). During this import process, Pink1 is cleaved within a predicted transmembrane segment by the rhomboid protease PARL (Pink1/Pgam5-associated rhomboid-like, originally termed Presenilin-associated rhomboid-like), resulting in a shortened form of Pink1 that undergoes retrotranslocation into the cytosol. The short form has an N-terminal phenylalanine that is recognized as a degron by the N-end rule and degraded by the 26S proteasome (120). Two other proteases, Yme1L and m-AAA, can also regulate Pink1 cleavage (119).

This normal process of Pink1 import and degradation is disrupted when mitochondria are damaged and results in outer membrane accumulation of Pink1, which acts to recruit Parkin to the mitochondria (119). In studies of the Pink1–Parkin pathway, the classic mitochondrial

Polyubiquitin: a chain of ubiquitin formed from the repeated conjugation of ubiquitin

stress used experimentally is depolarization of the inner membrane through uncoupling drugs such as carbonyl cyanide *m*-chlorophenyl hydrazine (CCCP), but stresses such as reactive oxygen species (ROS) generation or protein aggregation can also induce Pink1/Parkin-mediated mitophagy. CCCP treatment inhibits import of Pink1 through TIM and presumably results in lateral exit of Pink1 from TOM, with which it remains associated to form a high-molecular-weight complex on the outer membrane (119). How ROS and protein aggregation result in Pink1 activation remains to be clarified.

The accumulation of Pink1 on the outer membrane triggers a series of biochemical reactions that lead to mitophagy. Through transphosphorylation, Pink1 molecules autoactivate one another's kinase activity. In addition to autophosphorylation, a major target of Pink1 is serine 65 in ubiquitin (121, 122). Because there are E3 ubiquitin ligases that reside on the mitochondrial outer membrane, the mitochondrial surface has some level of basal ubiquitination that can act as a substrate for Pink1. Phosphorylated ubiquitin is recognized by Parkin, resulting in its recruitment to mitochondria and also its switch from an autoinhibited state to an activated, open conformation (123). This activated conformation is further stabilized by phosphorylation of the Ubl (ubiquitin-like) domain of Parkin by Pink1. Activated Parkin then ubiquitinates many mitochondrial proteins on the outer membrane and generates polyubiquitin chains of several linkages (64, 124, 125). These new ubiquitin molecules on the mitochondrial surface can be phosphorylated by Pink1, leading to a positive feedback loop that amplifies the mitophagy signal.

Polyubiquitination leads to recruitment of autophagy receptor proteins with ubiquitin binding and LC3-interacting regions (LIRs). The LIRs bind LC3 present on the surface of developing phagophores. The autophagy receptor proteins NDP52, OPTN, and TAX1BP1 have all been shown to facilitate Pink1/Parkin-mediated mitophagy (126). These receptors play partially redundant roles in recruiting the autophagosomal membranes to mitochondria, because single-receptor knockouts retain significant levels of mitophagy.

The finding that Pink1 and Parkin mediate stress-induced mitophagy has led to the proposal that these inherited forms of Parkinson's disease may result from a defect in mitochondrial quality control, ultimately leading to accumulation of dysfunctional mitochondria in the dopaminergic neurons that are most prominently affected in the disease. An important issue is whether this pathogenic process is also a factor in the more common, sporadic forms of Parkinson's disease. In addition, it remains to be clarified what types of mitophagy operate through a Pink1–Parkin mechanism. This is a pressing issue because studies in model organisms indicate that the Pink1–Parkin system is dispensable for basal mitophagy under unstressed conditions. In the mouse, the development of mitophagy reporter lines has facilitated the analysis of mitophagy in a broad range of cell types in vivo (127, 128). Remarkably, basal mitophagy is not decreased in neuronal and nonneuronal cells in *Pink1* knockout mice, including energetically intensive cells such as cardiomyocytes and skeletal myocytes (127). In fact, mitophagy is increased in pancreatic β cells, presumably because *Pink1* deficiency results in a mitochondrial dysfunction that activates a Pink1-independent mitophagy pathway (127). Similar conclusions were obtained from a fly model carrying a mitophagy reporter (129). However, another fly study showed that mitophagy increases with age, and that mitophagy in the flight muscles and dopaminergic neurons of 4-week-old flies is dependent on Pink1 and Parkin (130). The latter study indicates that some forms of basal mitophagy do require the Pink1–Parkin pathway in vivo. In future studies, these animal models will be valuable for determining whether some forms of stress-induced mitophagy are Pink1–Parkin dependent.

Although more research is required to catalog the types of mitophagy that require the Pink1–Parkin pathway, it has become clear that such processes have important physiological consequences in vivo. Mice lacking Parkin or Pink1 develop inflammation when subjected to exhaustive exercise (131). This inflammation occurs via the stimulator of interferon genes (STING) pathway,

an innate immunity program that upregulates cytokine release in response to intracellular DNA. On the basis of these data, it seems likely that certain types of stress, such as exhaustive exercise, generate damaged mitochondria that are removed by the Pink1–Parkin pathway. Consistent with this model, wild-type mice have upregulation of mitophagy in heart tissue after exhaustive exercise, and this response is muted in *Pink1* knockout mice (131). Because the STING innate immunity pathway is activated by cytosolic DNA, it is possible that failure to remove damaged mitochondria in *Pink1* mutant mice leads to exposure of mtDNA, its detection by the STING pathway, and induction of inflammation. Such inflammatory responses can lead to detrimental physiological effects. When *mutator* mice, which have an accumulation of mtDNA mutations, lack Parkin, they show neurodegeneration of dopaminergic neurons (132) and induction of an inflammatory response (131). Neuronal loss and upregulation of inflammatory cytokines do not occur when STING is removed (131). This result suggests that the dopaminergic neurons in this mouse model are dying from the inflammatory response, rather than from mitochondrial dysfunction itself.

In addition to causing autophagic degradation of mitochondria, the Pink1–Parkin pathway induces the formation of mitochondrial-derived vesicles (MDVs) that are targeted for degradation in the lysosome (133). The MDVs are vesicles ~70–100 nm in diameter that are enriched in oxidized cargo (134). They were initially identified as vesicles that target to peroxisomes, but now it is known that a subpopulation also targets to the lysosome. This population may provide an alternative pathway to remove damaged elements of mitochondria without disposing of the entire organelle. Note that mitophagy can also dispose of portions of mitochondria through a process whereby autophagosomes apparently pinch off fragments of mitochondria without using the canonical mitochondrial fission machinery (63). However, the Parkin-induced MDV pathway is distinct in that the core autophagy machinery, including ATG5, is dispensable (133). In addition, the pathway does not require depolarization of the mitochondrial inner membrane potential, which is the typical trigger for Parkin-mediated mitophagy. It has been proposed that the MDV pathway is an initial attempt to selectively remove only the damaged portions of mitochondria (133). When damage is more severe, the mitophagy pathway is triggered to remove the entire organelle.

Mutations Impairing Autophagy in Other Neurodegenerative Diseases

Several other proteins that function as adaptors to facilitate autophagy are mutated in neurodegenerative disease. Ubiquilin-2, SQSTM1/p62, and optineurin—adaptors that interact with LC3 and ubiquitin—have been found mutated in amyotrophic lateral sclerosis and frontotemporal lobar degeneration (135). These adaptors recruit a variety of cargo to autophagosomes, and mitochondria are among the cargos recognized by SQSTM1/p62 and optineurin. In addition, mutations in valosin-containing protein (VCP/p97) cause inclusion body myopathy associated with Paget disease and frontotemporal dementia. In fly models and patient cells, some VCP mutations are hyperactive and induce higher turnover of mitofusins, resulting in reduced mitochondrial fusion (136). In cell lines, the mitochondrial fusion and respiratory chain defects can be improved by administration of a p97 inhibitor.

Huntington's Disease

In addition to disease mutations in genes directly regulating mitochondrial fusion, fission, or mitophagy, mitochondrial dynamics has been implicated in neurological diseases in which the link is less direct. An example is Huntington's disease (HD), an autosomal dominant neurodegenerative disease associated with involuntary movements, rigidity, psychiatric features, and progressive cognitive decline (137). HD is caused by a CAG triplet nucleotide expansion in exon 1 of the *HTT*

(huntingtin) gene (138). Expansion of the CAG repeat to greater than 41 copies leads to fully penetrant disease, likely because the resulting polyglutamine stretch is highly prone to aggregation and has a toxic gain-of-function effect. Mutant Htt appears to have multiple cellular targets (139), including mitochondria (140), and affects many aspects of mitochondrial function, but this discussion focuses on its role in perturbing mitochondrial dynamics. Most studies consistently find that mutant Htt results in increased fragmentation of mitochondria due to aberrant fission. HD patient cells or cells overexpressing mutant Htt have mitochondrial fragmentation that has been attributed to Htt binding to Drp1 and enhancing its GTP-hydrolyzing activity (141, 142). Treatment of HD cell models with P110-TAT, a peptide designed to inhibit Drp1–Fis1 interactions (143), reduces mitochondrial fragmentation and improves cell viability (144). However, removal of Mff leads to exacerbation, rather than improvement, of the neurological and pathological features of an HD mouse model (145). Mitochondrial dynamics has also been implicated in Alzheimer's disease (AD) and has been reviewed elsewhere (146). In addition, a role for dysregulation of ER–mitochondrial contacts in Alzheimer's disease has been proposed (147). In the cases of HD, AD, and sporadic Parkinson's disease, a common issue has been whether the mitochondrial deficits observed play a primary role in pathogenesis or are secondary effects of another pathogenic process. Because these diseases result in widespread cellular stress, it is difficult to parse the contributions of the various pathologies, including mitochondrial dysfunction, to the clinical features.

Cancer and Mitochondrial Dynamics

Although neuronal cells appear to be frequently associated with strict requirements for mitochondrial dynamics, there is evidence that other cells, notably cancer cells, can be affected. Studies of lung cancer cells suggest that tumor phenotypes are associated with mitochondrial fragmentation (148) as well as higher expression of Drp1 and Drp1^{P616}, the phosphorylated form of Drp1 with increased activity. Interestingly, knockdown of Drp1 results in reduced proliferation and increased apoptosis in a lung cancer cell line, while increased Drp1 activity supports transformation driven by oncogenic Ras (149, 150). Activated Ras results in mitogen-activated protein kinase (MAPK) upregulation and leads to extracellular signal–related kinase 1/2 (ERK1/ERK2) phosphorylation of Drp1 at serine 616. Activation of MAPK signaling by oncogenic BRAF similarly causes activation of Drp1 (149), and Drp1^{P616} is highly associated with both dysplastic nevi and melanoma containing the oncogenic BRAF^{V600E} mutation, versus those containing wild-type BRAF (151). Inhibition of Drp1 activity is sufficient to reduce xenograft tumor growth driven by oncogenic Ras (150). Because of the pleiotropic physiological effects of mitochondrial dynamics, it is difficult to pinpoint the cellular processes important for tumor cell growth that are affected by changes in mitochondrial dynamics. Various studies have suggested that regulation of mitochondrial fusion or fission activity affects tumor metabolism, cell proliferation, cell migration, and maintenance of tumor stem cells (152).

CONCLUSIONS

Mitochondrial dynamics clearly plays an important role in regulating the function of these organelles. Some diseases, such as CMT2A, DOA, and some forms of intractable epilepsy, arise from a primary defect in mitochondrial fusion or fission. In addition, there are many other diseases in which mitochondrial dynamics is affected, but it remains unclear whether these defects are causative or secondary to another primary pathological process. This is an important issue to resolve in future research, because several major neurodegenerative diseases that affect large populations have associated problems in mitochondrial dynamics. Insights into the role of mitochondrial dynamics in pathogenesis have important therapeutic implications. In diseases associated with

excessive mitochondrial fission, it may be possible to alleviate disease by reducing mitochondrial fission. Alternatively, it may be possible to rebalance dynamics by increasing mitochondrial fusion.

SUMMARY POINTS

1. The dynamic behavior of mitochondria is critical for their function, providing benefits in regulation of morphology, content exchange, maintenance of mtDNA and OXPHOS activity, equitable inheritance of mitochondria during cell division, and degradation of damaged mitochondria.
2. The key mediators of mitochondrial fusion and fission belong to the dynamin superfamily of GTPases, which use GTP hydrolysis to perform mechanical work on lipid bilayers.
3. Several neurodegenerative diseases are caused by mutations in genes directly involved in mitochondrial fusion, fission, and degradation.
4. Some common diseases, including neurodegenerative diseases and some cancers, are associated with defects in mitochondrial dynamics, but more research is required to determine if these defects play a causative role.

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Errata

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