[Frontiers in Bioscience, Landmark, 20, 229-246, January 1, 2015]

Morphological control of mitochondrial bioenergetics

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1. ABSTRACT

The major function of mitochondria is production and supply of cellular energy. Mitochondria are highly dynamic organelles undergoing frequent shape changes via fission and fusion. Many studies have elucidated the molecular components mediating fission and fusion and their regulatory mechanisms, and mitochondrial shape change is now recognized as an essential cellular process that is closely associated with functional states of mitochondria. This review updates the recent advancements in fission and fusion mechanisms, and discusses the bi-directional relationship between mitochondrial morphology and energetic states in physio-pathological settings.

2. INTRODUCTION

Synthesis of ATP from fuel oxidation is the main function of mitochondria. Additionally, mitochondria play a critical role in heme and steroid biosynthesis as well as regulations of cellular redox, Ca^{2+}, and cell death. By doing so, mitochondria are involved in virtually every aspect of cell function; therefore, it is no surprise that mitochondrial dysfunction is associated with many human diseases. Mitochondria carry out these diverse functions within their highly compartmentalized structure organized in the unique double membrane system. The outer mitochondrial membrane envelops the entire organelle whereas the matrix-enclosing inner membrane is organized into two morphologically distinct but contiguous subregions of inner boundary membrane and cristae. Respiratory complexes and ATP synthases reside mostly in the crista membrane.

While the internal organization of mitochondria is defined to some degree, the exterior appearance of mitochondria within the cytoplasm is quite diverse, ranging from small vesicles to large blobs or reticular networks, depending on cell types and their functional status. Through live cell observations of mitochondria in culture, one can readily appreciate the dynamic nature of mitochondrial morphology and distribution. Morphological changes

observable in mitochondria include fission/fusion, extension/retraction, and branching/debranching to list a few (1-3). Among these different types of shape change, fission and fusion of mitochondria are the most characterized and studied processes. The molecular components mediating fission and fusion have been identified and, more recently, regulatory mechanisms for mitochondrial fission and fusion have been reported in different pathological and environmental conditions. Growing evidence indicates that morphological control of mitochondria is an essential cellular process, necessary not only for cell survival, but also for efficient energy metabolism in changing environments (4). This review focuses on the functional significance of mitochondrial shape change mediated by fission and fusion in a physio-pathological context. Many excellent reviews are available for detailed mechanisms of mitochondrial fission and fusion. Although it may be somewhat redundant, we update the fission and fusion mechanisms in the first part of this review. The second part will discuss the correlation between mitochondrial morphology and bioenergetic status.

3. MOLECULAR MECHANISMS OF MITOCHONDRIAL FISSION AND FUSION

The balance between mitochondrial fission and fusion determines the overall morphology of the mitochondrial network. Membrane remodeling proteins belonging to the dynamin family of large GTPases mediate mitochondrial fission and fusion. In mammals, these proteins are dynamin-like/related protein 1 (DLP1/Drp1), mitofusin isoforms (Mfn1 and Mfn2), and optic atrophy 1 (OPA1). DLP1 is a cytosolic protein that translocates to mitochondria for fission whereas Mfns and OPA1 are associated with the mitochondrial outer and inner membrane, respectively, and mediate fusion. Many pathophysiological conditions alter mitochondrial morphology by regulating fission and fusion proteins. Therefore, while the focal point of this review is the metabolic and physiological roles of mitochondrial morphology, we will first briefly describe how mitochondrial fission and fusion take place and are regulated at the molecular level.

3.1. Mitochondrial fission

3.1.1. Mitochondrial fission by DLP1 and its receptors

DLP1 is the main protein mediating mitochondrial fission. As mentioned, DLP1 is a member of the dynamin GTPases known to have a mechanical activity that pinches lipid membranes (5, 6). Conventional dynamin has been shown to self-assemble to form spirals that wrap around and sever the lipid tubule through GTP hydrolysis (7, 8). Similar activities have been reported with DLP1 and the yeast homologue Dnm1 (9-12). Cryo-electron microscopy showed that GTP hydrolysis induces two-fold reduction of the diameter of Dnm1-coated lipid tubules *in vitro*, suggesting that Dnm1 can generate a contractile force that constricts the membrane to mediate mitochondrial fission (12). During mitochondrial fission in cells, DLP1 in the cytosol becomes associated with mitochondria by interacting with receptor molecules. Several resident mitochondrial outer membrane proteins bind to DLP1 and are suggested to be DLP1 receptors. These proteins include Fission 1 (Fis1), Mitochondrial fission factor (Mff), Mitochondrial dynamics protein of 49 kDa and 51 kDa (MiD49/MiD51), and Mitochondrial elongation factor 1 (MIEF1, identical to MiD51).

Fis1 is anchored at the mitochondrial outer membrane and binds to DLP1 (13, 14). The role of Fis1 in mitochondrial fission is supported by studies showing that overexpression of Fis1 results in DLP1-mediated mitochondrial fragmentation, and that inhibition of Fis1 leads to mitochondrial elongation (13-16). However, Fis1 distributes evenly throughout the mitochondrial tubule and is not colocalized with DLP1 puncta on the mitochondrial surface (16, 17). Furthermore, overexpression or deletion of Fis1 does not appear to affect the DLP1-mitochondria association (17, 18), raising the question of whether Fis1 is a *bona fide* receptor for DLP1. Another mitochondrial outer membrane protein, Mff, was identified to function in mitochondrial fission (19). Additional studies showed that knockdown of Mff reduces DLP1 association with mitochondria and induces mitochondrial elongation, whereas overexpression of Mff enhances DLP1 translocation to mitochondria, causing mitochondrial fragmentation (20). Furthermore, an artificial targeting of Mff to the plasma membrane redirected DLP1 to this new location, indicating that Mff recruits DLP1 (20). MiD49 and MiD51/MIEF1, have also been shown to bind and recruit DLP1 to mitochondria (21, 22). However, unlike Mff and Fis1, MiD49 and 51 act as a fission inhibitory factor by sequestering DLP1 from the fission sites; hence, their overexpression induces mitochondrial elongation (23). As discussed, Fis1, Mff, MiD49, and MiD51 bind to DLP1 and may act as DLP1 receptors. A recent study using null cell lines for Fis1 and/or Mff indicated that Fis1 and Mff contribute to mitochondrial

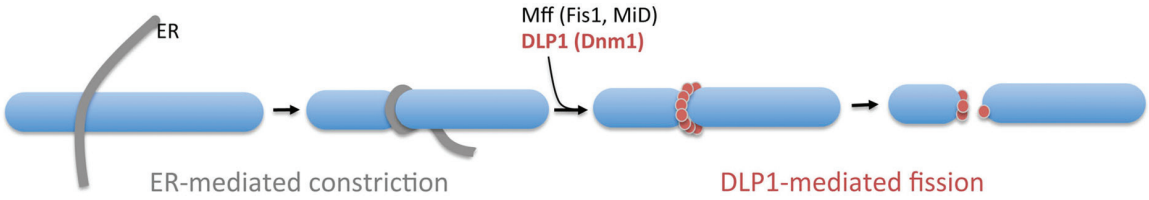

Figure 1. Mitochondrial fission. ER tubule has been shown to wrap around mitochondria for initial constriction of the tubule. DLP1/Dnm1 is recruited to the constricted region by Mff and mediates fission.

localization of DLP1 independently of each other with Mff being more dominant (24). In addition, MiD49 and MiD51-mediated DLP1 recruitment is independent of Mff and Fis1 (23, 24). Further studies will elucidate whether these proteins coordinate or compete for controlling mitochondrial morphology, possibly varying in different pathophysiological conditions.

Close contact between the endoplasmic reticulum (ER) and mitochondria has been known to exist for some time, and recent studies indicate that the ER plays a role in mitochondrial fission. ER tubules were observed to wrap around and constrict mitochondrial tubules, marking sites for DLP1 recruitment for fission (25) (Figure 1). The ER-mediated mitochondrial constriction is an initial event occurring independently of Mff and DLP1 (25). In budding yeast, the multi-protein complex called ER-mitochondria encounter structure (ERMES) (26) has been shown to participate in ER-mediated mitochondrial fission (27), although a structure homologous to ERMES is not found in mammals. In another study, the ER-associated formin INF2 was shown to promote actin assembly at the ER-mitochondria contact, driving mitochondrial constriction (28). Actin polymerization was also suggested to be involved in mitochondrial fragmentation induced by *Listeria* toxin listeriolysin (LLO) (29, 30). Interestingly, LLO-induced mitochondrial fission was observed at ER crossing sites, suggesting the participation of ER-mediated mitochondrial fission although it is independent of DLP1 and Mff (29). ER-mitochondria contacts have important roles in cell and organ physiology through lipid exchange, Ca^{2+} transfer, and apoptotic regulation (31-34). Participation of the ER in controlling mitochondrial morphology suggests an important role of mitochondrial shape in physiological processes mediated through the ER-mitochondrial contacts.

3.1.2. Regulation of mitochondrial fission through DLP1 phosphorylation

Mitochondrial fission has been shown to be regulated at multiple levels: gene expression, proteasomal degradation, phosphorylation, sumoylation, S-nitrosylation, and O-glycosylation (35-44). Because several metabolic and pathologic insults have been shown to alter mitochondrial morphology through DLP1 phosphorylation-dephosphorylation, we will discuss DLP1 phosphorylation-mediated regulation of mitochondrial fission. Two serine residues at the tail region of DLP1 have been found to be phosphorylated by multiple different kinases.

The upstream site (--IMPA**S**PQKG--) is phosphorylated by CDK1, PKCδ αvδ ERK1/2 (45-47). Phosphorylation of DLP1 at this site has been shown to increase fission, regardless of the phosphorylating kinase. At the onset of mitosis, CDK1 phosphorylates DLP1 and induces mitochondrial fragmentation, which facilitate even distribution of mitochondria to daughter cells (45). Further study identified the mitotic kinase Aurora A as initiating the signaling cascade in which the small GTPase RALA and its effector RALBP1 bring DLP1 and CDK1 to mitochondria for DLP1 phosphorylation and mitochondrial fission (48). Upon exit from mitosis, DLP1 has been found to be degraded rather than dephosphorylated in order to restore tubular mitochondrial morphology. DLP1 degradation was found to be mediated by a cell cycle E3 ubiquitin ligase, APC/C (anaphase-promoting complex/cyclosome) (41). PKCδ phosphorylates DLP1 at Ser_{CDK1}, which is associated with mitochondrial fragmentation and cell death in oxidative stress and hypertension (46). ERK1/2 was also suggested to phosphorylate Ser_{CDK1}, increasing mitochondrial fission in high glucose incubation (47).

The serine residue 20-amino acids downstream from the CDK1 site is within the cyclic AMP-dependent protein kinase (PKA) consensus motif (--VA**RKLS**AR--) and can be phosphorylated by PKA, calcium/calmodulin-dependent protein kinase Iα (CaMKIα), and Rho-associated coiled coil-containing protein kinase 1 (ROCK1) (49-52).

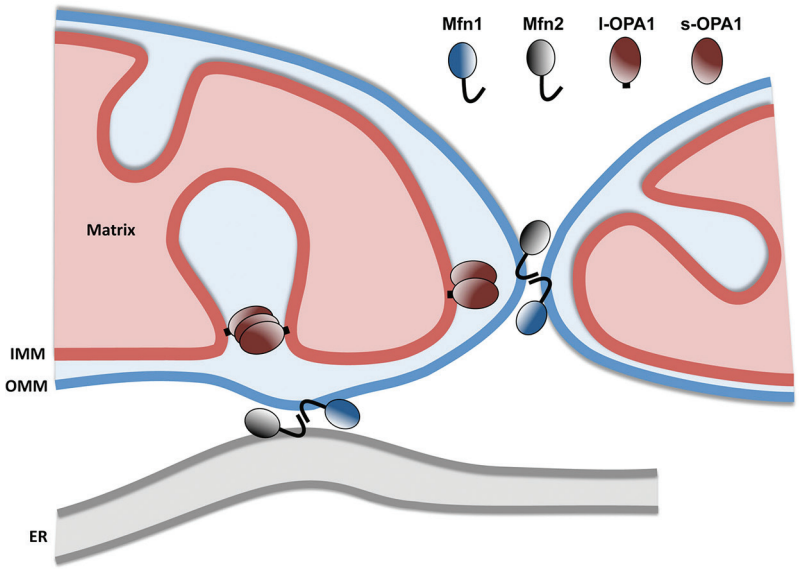

Figure 2. Mfn and OPA1. Mitochondrial fusion is initiated by tethering of fusing mitochondria through interactions between Mfns. Two isoforms, Mfn1 and Mfn2, can form homotypic and heterotypic complexes. Mfn2 also localizes to the ER membrane where it interacts with mitochondrial Mfn1 and Mfn2 to mediate tethering between the ER and mitochondria. OPA1 mediates inner membrane fusion. The presence of both long and short forms of OPA1 is necessary for fusion. OPA1 is also associated with cristae junction and regulates cristae structure.

In contrast to the phosphorylation at Ser_{CDK1}, which induces mitochondrial fission, phosphorylation at this serine residue (Ser_{PKA}) results in different outcomes depending on the phosphorylating kinase. DLP1 phosphorylation by PKA induces mitochondrial elongation by decreasing DLP1 GTPase activity and mitochondrial association (49). PKA-mediated DLP1 phosphorylation occurred under starvation, stress, exercise, and β-adrenergic activation (50, 53). PKA translocates to mitochondria by binding to the A kinase anchoring protein 1 (AKAP1, also known as AKAP121) (50) whose level is controlled by the ubiquitin ligase Siah2 (54). It has been shown that, under hypoxic conditions, Siah2-mediated degradation of AKAP1 not only decreases DLP1 phosphorylation by PKA but also enhances DLP1-Fis1 interaction, thus increasing mitochondrial fission (54). The Ca^{2+}-dependent phosphatase calcineurin and protein phosphatase 2A (PP2A) both dephosphorylate the PKA-phosphorylated DLP1 to increase mitochondrial fission (50, 55-57). In addition, the mitochondrial phosphoglycerate mutase/protein phosphatase, PGAM5, also dephosphorylates DLP1 at the Ser_{PKA} during necrotic cell death, resulting in mitochondrial fragmentation (58). In contrast to the inhibitory effect of PKA phosphorylation on the DLP1 function, CaMK1α phosphorylate DLP1 at the Ser_{PKA} upon Ca^{2+} influx in neurons and increase mitochondrial fission by enhancing Fis1-DLP1 interaction (51). Additionally, ROCK1 phosphorylated DLP1 at Ser_{PKA} under hyperglycemic insult in kidney podocytes and endothelial cells, also resulting in increased mitochondrial fission.

3.2. Mitochondrial fusion

3.2.1. Mitochondrial fusion by Mfn and OPA1

The dynamin-related protein mitofusin mediates fusion of the outer mitochondrial membranes. Mammals have two mitofusin isoforms, Mfn1 and Mfn2. Transcriptional analyses indicate that Mfn1 is more generally expressed among different tissues whereas Mfn2 shows high expression in heart and skeletal muscle (59). On the other hand, at the protein level, Mfn1 is abundant in heart and testis whereas Mfn2 is predominant in the brain (60). Mfns are anchored at the mitochondrial outer membrane through two transmembrane domains near the C-terminus. The Mfn molecule spans the outer membrane twice, leaving the bulk of the Mfn molecule exposed to the cytosol (61, 62). Mfn-mediated outer membrane fusion is initiated by tethering of apposing mitochondria through interaction between their Mfn molecules (62) (Figure 2). Therefore, the presence of Mfn in each of the two fusing membranes is prerequisite for outer membrane fusion (62). Mfn1 and Mfn2 can form both homotypic and heterotypic

complexes (63). *In vitro* studies indicated that Mfn1 has 8-fold higher GTPase activity than Mfn2, and that tethering by Mfn1 homotypic interaction is more efficient than that by the interaction between Mfn2 (64). However, for actual fusion efficiency, Mfn1-Mfn2 heterotypic trans complex showed higher rates of fusion compared to the homotypic complex (65). Crystal structure of the Mfn1 tethering complex revealed an approximately 16 nm gap between the opposing membranes (62), which is too far apart for lipid bilayer mixing. It is likely that the GTPase activity of Mfn would bring the two membranes closer for fusion. Further studies are necessary to elucidate whether Mfn has mechanical activity similar to other dynamin proteins, potentially via a GTP hydrolysis-induced conformational change.

OPA1 is another dynamin family protein functioning in mitochondrial membrane remodeling. OPA1 has the N-terminal mitochondrial transit sequence followed by a transmembrane domain that anchors the protein at the inner membrane. Thus, OPA1 faces the inter-membrane space (Figure 2). In addition to alternative splicing that produces 8 different splice variants (66), OPA1 undergoes proteolytic cleavage events within the two exons downstream of the transmembrane domain (67, 68). This proteolytic cleavage generates transmembrane region-free soluble forms of OPA1, which remain associated with the inner membrane. Analyses with different splice variants show that the presence of both the short cleaved form and the transmembrane-containing long form of OPA1 are necessary for mitochondrial fusion (68). OPA1 has been shown to have the capacity to constrict membranes, as recombinant soluble OPA1 can form tubular protrusions on liposomes (69). Unlike Mfn, the presence of OPA1 at only one of the fusing membranes is sufficient for mitochondrial fusion, suggesting two different mechanisms for outer and inner membrane fusion (70). Completion of mitochondrial fusion requires sequential fusion of outer and inner membranes through the highly coordinated actions of Mfn and OPA1 due to the presence of two distinct membrane systems. While a linker protein interacting with both outer and inner membrane fusion proteins was identified to coordinate mitochondrial fusion in yeast (71), the mechanisms of the coordinated fusion of outer and inner membranes are not yet fully understood in the mammalian system.

3.2.2. More on Mfn and OPA1

While it is well established that Mfn and OPA1 are mitochondrial fusion proteins, additional functions of these proteins have also been reported. Mfn2 was found to localize to the ER membrane where it interacts with mitochondrial Mfn1 and Mfn2 to mediate tethering between the ER and mitochondria (33) (Figure 2). The Mfn2-mediated ER-mitochondria tethering facilitates efficient Ca^{2+} transfer between these two organelles (33). OPA1 deficiency has been shown to cause disruption of cristae structure (72, 73), suggesting it may have an additional function other than inner membrane fusion (74). OPA1 appears to be localized to the cristae junction (Figure 2) and is proposed to control cristae remodeling for efficient release of cytochrome *c* during apoptosis (74). A recent study suggests that OPA1 plays a role in respiratory complex assembly and stability by controlling cristae shape (75).

Early on, the mitochondrial inner membrane potential was found to be required for mitochondrial fusion (76, 77). It turns out that the membrane potential regulates the aforementioned proteolytic cleavage of OPA1 (67, 68, 78). Inner membrane-associated proteases including PARL (presenilin-associated rhomboid-like), i-AAA (Yme1L), m-AAA (paraplegin, AFG3L1 and 2), and the inner membrane metalloprotease OMA1 all mediate OPA1 cleavage (67, 68, 79-83). Among those, paraplegin and OMA1 were shown to cleave OPA1 upon mitochondrial depolarization (67, 83). OPA1 cleavage in depolarized mitochondria is thought to serve a quality control function, preventing dysfunctional mitochondria from fusion, by which a healthy mitochondrial population is maintained (84).

Proteasomal degradation also regulates mitochondrial fusion by modulating the level of Mfn. The mitochondrial E3 ubiquitin ligase MARCH5/MITOL was reported to regulate mitochondrial morphology through ubiquitination of not only Mfn1 and 2, but also of fission proteins DLP1 and Fis1 (42, 43, 85, 86). A recent study, however, indicates that MITOL ubiquitinates mitochondrial Mfn2 without proteasomal degradation and facilitates the Mfn2-mediated ER-mitochondria tethering (87). Another E3 ligase, the Parkinson's disease-associated cytosolic E3 ligase Parkin, binds to depolarized mitochondria and ubiquitinates Mfn1 and Mfn2 (88, 89). Subsequent proteasomal degradation of Mfns prevents fusion of dysfunctional mitochondria, allowing their autophagic removal. Parkin was shown to be recruited to depolarized mitochondria by binding to Mfn2 in a PINK1-dependent manner (90). Parkin binds to PINK1-phosphorylated Mfn2 at Thr111 and Ser442 and ubiquitinates Mfn2 (90). In addition, another Mfn2 phosphorylation has been found

to be associated with ubiquitylation-proteasomal system (91). The MAP kinase JNK phosphorylates Mfn2 at Ser27 under stress conditions, which promotes selective degradation of Mfn2 through the HECT domain ubiquitin ligase Huwe1 (91). Huwe1 binds to Ser27-phosphorylated Mfn2 and degrades Mfn2, which promotes mitochondrial fragmentation and apoptosis under condition of stress (91).

Recognizing and eliminating depolarized/dysfunctional mitochondria are cellular processes important for maintaining cell and organ function. As just discussed, the cell's mitochondrial fusion machinery is a critical system that recognizes bioenergetically impaired mitochondria in order to segregate them from the dynamic network of functional mitochondria and target them for autophagic removal. Mitochondrial fission can separate and generate small dysfunctional mitochondria destined for autophagy. In this context, mitochondrial morphology regulated by fission and fusion must have an important function in maintaining and controlling bioenergetic activity of mitochondria. Indeed, evidence indicates that mitochondrial morphology and function influence one another bi-directionally.

4. MITOCHONDRIAL BIOENERGETICS AND MORPHOLOGY

4.1. Mitochondrial bioenergetics – a simplistic view

Mitochondria produce ATP through oxidative phosphorylation (OXPHOS). In its essence, OXPHOS is the coupled event of nutrient oxidation and phosphorylation of ADP. A complete oxidation of nutrients generates CO_2 along with the reducing equivalents NADH and $FADH_2$ which donate electrons to the electron transport chain (ETC). A series of redox reactions through the ETC complexes generates the proton motive force with which ATP synthase phosphorylates ADP to produce ATP, while molecular oxygen serves as the terminal electron acceptor to produce H_2O (92, 93).

OXPHOS reactions occur within the unique structural organization of mitochondria, which facilitates efficient ATP production. NADH and $FADH_2$ generated from the Krebs cycle in the mitochondrial matrix pass electrons to the ETC complexes (Complexes I – V) that are embedded in the inner (cristal) membrane. Electrons from NADH are transferred to Complex I (NADH-Coenzyme Q reductase, or NADH-dehydrogenase) and flow down to Complexes III (Coenzyme Q-cytochrome *c* reductase) through the inner-membrane associated mobile electron carrier ubiquinone, then subsequently to Complex IV (cytochrome *c* oxidase) via cytochrome *c*. The ETC Complex II (Succinate-Coenzyme Q reductase) is the succinate dehydrogenase of the Krebs cycle, which generates $FADH_2$ and passes electrons down to ubiquinone and Complexes III and IV. Complexes I, III, and IV are the proton pumps, taking matrix protons and releasing them into the inter membrane space as a result of electron transfer reactions. This proton pumping during electron transfer creates the proton motive force across the inner membrane, which provides the energy for ATP synthesis by Complex V (ATP synthase or F_oF_1- ATPase).

The proton motive force (Δp) is an electrochemical gradient with both electrical (membrane potential, $\Delta\Psi$) and chemical (proton gradient, ΔpH) components. With the well-sealed inner membrane, ATP is synthesized when protons flow down the electrochemical gradient back to the matrix through the ATP synthase. The OXPHOS coupling is the efficiency in using the proton gradient for ATP synthesis. However, when protons bypass ATP synthase and flow back to the matrix by other means, the coupling of oxidation to phosphorylation becomes "uncoupled". Dinitrophenol, CCCP, and FCCP, which have protonophoric activity, are known chemical uncouplers. In addition, a class of carrier proteins called uncoupling proteins (UCPs) can transport protons at the mitochondrial inner membrane (94, 95).

Although OXPHOS is an efficient ATP production mechanism, some electrons slip out of the ETC and react with oxygen to produce superoxide (96-98). Superoxide is rapidly converted to hydrogen peroxide by superoxide dismutase (99). Both superoxide and hydrogen peroxide are reactive oxygen species (ROS) that have signaling function at physiological concentrations, but are harmful when present in excess. ROS production is more pronounced when electron transfer becomes slow, which occurs with ETC complex dysfunction as well as in mitochondrial hyperpolarization (97, 100). When the ETC is functionally intact, uncoupling relieves hyperpolarization and accelerates electron transfer, decreasing ROS production (101, 102). Superoxide has been shown to increase uncoupling by activating the uncoupling proteins of the inner membrane (103, 104), a self-protective mechanism preventing ROS overproduction in mitochondria.

4.2. Mitochondrial morphology and energetic states: bi-directional influence

Several observations have demonstrated that changes in the bioenergetic state alter mitochondrial morphology. Most notably, OXPHOS inhibition by ETC poisons have been shown to disrupt normal mitochondrial morphology (105-107). Rotenone and antimycin A, inhibitors of Complex I and III, respectively, caused mitochondrial fragmentation in many cell types (106, 108). Inhibition of Complex II by 3-nitropropionic acid (3-NP) induced mitochondrial swelling and fragmentation possibly through an increased level of ROS (107). Additionally, chemical uncouplers also induced mitochondrial fragmentation (76, 77, 109). Similar to uncouplers, inhibition of the ETC generally causes a loss of inner membrane potential. As described above, mitochondrial depolarization induces the cleavage of OPA1 molecules to prevent mitochondrial fusion, thus mitochondria become fragmented (67, 68, 78). Morphological fragmentation of depolarized mitochondria is important from a cellular energetic perspective, as it facilitates the selective removal of dysfunctional mitochondria (84). The morphological alterations following OXPHOS inhibition indicate that mitochondrial morphology reflects the bioenergetic states of mitochondria.

Conversely, disrupting mitochondrial morphology by genetic manipulation of fission and fusion alters mitochondrial function. Fragmented mitochondria in cells lacking both Mfn1 and Mfn2 showed reduced respiration (110). Similar mitochondrial dysfunction was observed with OPA1 silencing, indicating that inhibition of mitochondrial fusion causes a functional defect in mitochondria (110). On the other hand, there are conflicting observations in terms of functional alterations upon disruption of mitochondrial fission. Silencing DLP1/Drp1 in HeLa cells decreased membrane potential, respiration, ATP levels, and cell proliferation, which was accompanied by increased ROS levels, oxidative damage, mitochondrial DNA loss, and enhanced mitophagy (105, 111). In contrast to these severe functional defects observed from DLP1 silencing in HeLa cells, DLP1-knockout mouse embryonic fibroblasts showed no defects in inner membrane potential, respiration, ATP content, mitochondrial DNA, or autophagy (112). While the mechanistic aspect of the effect of fission inhibition on mitochondrial function will be discussed later, it is evident that there is a bi-directional relationship between mitochondrial morphology and bioenergetic activity.

4.3. Mitochondrial morphology and functionality in physio-pathological settings

It has been observed that mitochondria were fragmented under hypoxic and anoxic conditions, and that inhibiting mitochondrial fragmentation decreased cardiac ischemic injury in mice (54, 113). Hypoxia-induced mitochondrial fragmentation is mediated by PKA, its mitochondrial receptor AKAP121, and Fis1. Hypoxia induces proteasomal degradation of AKAP121, which decreases PKA phosphorylation of DLP1 and increases Fis1-DLP1 interaction, which leads to mitochondrial fragmentation (54). Hence, inhibiting mitochondrial fragmentation in mice by blocking proteasomal degradation of AKAP121, or by treating with a DLP1 chemical inhibitor, decreased cardiac ischemic or ischemia-reperfusion injury, showing smaller infarct area compared to wild type mice (54, 113).

The metabolic insult of hyperglycemic or high glucose-high fat conditions also induces mitochondrial fragmentation and is associated with ROS increase (114-117). Hyperglycemia has been shown to activate mitochondrial fission through DLP1 phosphorylation by ROCK1 and ERK1/2 (47, 52). *In vivo*, ROCK1 knockout mice showed low ROS levels as well as less mitochondrial fragmentation and decreased apoptosis in kidney glomeruli under diabetic conditions (52). Furthermore, direct inhibition of mitochondrial fission in mice by the dominant-negative fission mutant DLP1-K38A decreased ROS levels and oxidative stress as well as improved renal function in diabetic kidneys (118). In addition, DLP1 knockdown in rat brain acutely infused with a glucose bolus also decreased the ROS levels in hypothalamus (119).

Contrastingly, cellular stresses including inhibition of transcription or translation, UV irradiation, and serum and amino acid depletion have been shown to induce mitochondrial elongation, and was called stress-induced mitochondria hyperfusion (SIMH) (120). Mfn1 and OPA1-mediated fusion is required for SIMH. In addition, stomatin-like protein 2 (SLP-2) protects OPA1 from proteolytic cleavage, maintaining the fusion activity of OPA1 during SIMH. Increased OXPHOS and ATP production were observed in SIMH, suggesting that SIMH is a pro-survival mechanism for coping with cellular stresses (120). Similar to SIMH, nutrient depletion also induces mitochondrial elongation (53, 121). This starvation-induced mitochondrial elongation was found to be the consequence of DLP1

phosphorylation by PKA and dephosphorylation at the upstream Ser_{CDK1} (53, 121), which decreases the fission activity of DLP1. The unopposed fusion events mediated by Mfn and OPA1 induce mitochondrial elongation during nutrient depletion. Mitochondria in cells lacking Mfns or OPA1 remained fragmented during nutrient deprivation and underwent an ATP depletion-driven cell death. Elongated mitochondria in nutrient starvation showed expansion of cristae surface and an increase of ATP synthase dimerization, indicating increased OXPHOS, similar to SIMH. Hence, mitochondria in elongated form were able to maintain their ATP producing activity during nutrient starvation and, at the same time, were protected from autophagy (53).

Recently, mitochondrial fission and fusion in hypothalamic neurons have been found to play an important role in integrative physiology of whole body energy metabolism. The hypothalamus of the central nervous system regulates systemic energy homeostasis through the coordination of food intake and energy expenditure. Glucose-responsive neurons in the ventromedial hypothalamic area (VMH) regulate food intake as well as insulin secretion. Glucose infusion to the brain through intracarotid injections in rats showed rapid recruitment of DLP1 to mitochondria and ROS increase in the VMH, as well as an increase in insulin secretion and a decrease in food intake after fasting (119). Silencing DLP1 in the VMH blunted the glucose-induced ROS increase, insulin secretion, and satiation, suggesting that mitochondrial fission is a key mechanism regulating hypothalamic glucose sensing. In addition to the VMH, the nearby arcuate nucleus contains agouti-related protein (Agrp) neurons and pro-opiomelanocortin (POMC) neurons that also sense circulating nutrients (e.g. glucose and fatty acids) and hormones (e.g. insulin and leptin). These two types of neurons exert opposite effects on metabolism and feeding behavior: activation of Agrp neurons stimulates appetite, as does inhibition of the POMC-releasing neurons (122, 123). In a recent report, it was shown that high fat diet-induced obese mice displayed disruptions of both mitochondrial morphology and ER-mitochondrial contacts in POMC neurons through reduced expression of Mfn2 (124). Overexpression of Mfn2 in the arcuate nucleus attenuated these alterations along with a decrease of body weight and ER stress on a high fat diet. Conversely, knockout of Mfn2 in POMC neurons induced obesity through ER stress-induced leptin resistance, suggesting that Mfn2 controls systemic energy balance by mediating ER-mitochondria contacts and thus ER stress (124). On the other hand, knockout of Mfn1 or Mfn2 in Agrp neurons had no effect on ER-mitochondria contact and ER stress on either normal or high fat diet (125). Instead, mitochondria in Agrp neurons underwent fusion in high fat diet, and preventing mitochondrial fusion resulted in diminished weight gain and fat mass in high fat diet. It was found that Mfn1- or Mfn2-knockout Agrp neurons showed a decreased electric activity in high fat diet, but not in normal diet. This decreased firing in Mfn knockout Agrp neurons was normalized when the intracellular ATP level in Mfn knockout neurons was made equal to that of control neurons, indicating that lack of mitochondrial fusion in Agrp neurons causes ATP deficiency and thereby decreased electrical activity. These findings demonstrate that mitochondrial dynamics are key factors in systemic regulation of energy homeostasis by modulating morphological plasticity as well as ER-mitochondria contacts in response to a changing metabolic environment.

4.4. Control of respiration coupling: a mechanism linking mitochondrial morphology and bioenergetic activity?

As discussed, many studies have found specific regulations of mitochondrial fission and fusion under certain stress or insult conditions. What is unknown, however, is whether there are overarching principles by which mitochondrial morphology and the correlative bioenergetic state can be defined. One potential clue can be found in the transient change in mitochondrial morphology and ROS levels during high glucose stimulation. In cultured cells, acute hyperglycemic incubation induces transient shortening of mitochondria, in which initial rapid formation of small and shorter mitochondria through fission was followed by recovery of longer tubular morphology within an hour. Interestingly, superoxide levels also increased and decreased in high glucose conditions. The temporal profile of superoxide levels in acute high glucose incubation was nearly identical to that of morphological change, suggesting a close relationship between mitochondrial ROS production and mitochondrial morphology (116). Importantly, decreasing mitochondrial fission normalized the ROS level, both in cultured cells and in animals under hyperglycemic conditions (52, 115, 116, 118, 119), indicating that mitochondrial fission plays an important role in ROS production in high glucose conditions. Because the ETC is the main source of ROS production in hyperglycemia, these observations suggest that mitochondrial fission may control ETC activity. One

underlying mechanism for mitochondrial fission regulating the ETC was found to be the modulation of inner membrane proton leak (118). In fission deficient cells, large interconnected mitochondria were found to lose and recover their inner membrane potential repeatedly, which was manifested in respiration measurements as increased proton leak (uncoupling) (118). Hence, inducing mild uncoupling in mice by a low-level expression of DLP1-K38A decreased ROS and oxidative stress in hyperglycemia (118). Inhibition of mitochondrial fission in a pancreatic β-cell line was also found to increase proton leak, which, in turn, prevented ATP augmentation in glucose stimulation and thus blocked the insulin secretion (126). In another study described in the previous section, DLP1 silencing in glucose-infused hypothalamus was also shown to increase proton leak and diminish mitochondrial ROS level along with ROS-mediated downstream metabolic signaling (119).

Because inhibition of mitochondrial fission increases mitochondrial size, these observations along with numerous results showing fission inhibition abrogating ROS increase brings up an intriguing possibility that the length/size of mitochondria may be an important factor for respiration coupling. One potential mechanism for increased uncoupling in larger and longer mitochondria may involve transient permeability transition (tPT). The tPT has been shown to occur normally in individual mitochondria (127, 128), which has minimal effect on overall cellular mitochondrial function because of its locally restricted nature. However, when mitochondria become longer and interconnected, the same tPT would affect all connected mitochondria and amplify its depolarizing effect, exhibiting respiration uncoupling (118). A yet unidentified mechanism, rather than conventional protein channels/pores, could be involved in this fission deficiency-induced proton leak (118). This premise argues that, as mitochondrial interconnection increases, so does respiration uncoupling. It is possible that the balanced mitochondrial fission and fusion in normal conditions represent limited shifts between partially coupled and partially uncoupled states. An increase in metabolic flux may shift the balance to fission, producing tightly coupled mitochondria with short and small morphology. Intriguingly, small and short mitochondrial appearance here may represent the Hackenbrock's "condensed" internal conformation observed with coupled respiration in isolated mitochondria (129). Continuous metabolic excess induces hyperpolarization and ROS increase, which may evoke a signal to shift toward fusion, increasing mitochondrial size to adjust ROS and ATP production by decreasing respiration coupling. It should be noted here that dynamic reversible transition from one form of mitochondria to another is likely a physiological cellular response, as opposed to an irreversible terminal change of mitochondrial morphology such as the fragmented dysfunctional mitochondria associated with cell death and mitophagy, for which the mitochondrial size-coupling correlation may not be applicable. In addition, the notion of longer mitochondria being less coupled appears to be in contradiction with other observations that mitochondrial elongation is associated with enhanced ATP production (53, 120, 130), which is presumed to require coupled mitochondria, although respiration coupling was not specifically evaluated in these studies. It is possible that there are mitochondrial size thresholds for different coupling states, which may further be dependent on the nature of stress or stimulation as well as cell type-specific mitochondrial morphology. Nevertheless, the potential mitochondrial size-respiration coupling correlation described for fission manipulation in high glucose stimulation would be an attractive postulation with which certain mitochondrial morphologies and their corresponding bioenergetic states can be defined. Further investigation is necessary to evaluate whether the correlation between mitochondrial size and coupling state is valid in other stresses or morphological manipulations.

5. CONCLUSION

Since the first report on one of the dynamin-related proteins functioning in control of mitochondrial morphology, mitochondrial dynamics has drawn a great deal of attention due to its involvement in apoptosis and many human pathologies. The structure-function relationship exists at every level of the biological system, from simple molecules to the complex organization of cells, tissues, and organisms. Selection pressure throughout the long evolutionary process found the most efficient structures and forms necessary for their respective functions. While the elaborate internal organization of mitochondria attests to their structural adaptation for efficient ATP production, the functional significance of the dynamic nature of overall mitochondrial morphology is not well understood. Considering that the most important function of mitochondria is ATP production, their bioenergetic activity must be a crucial factor for dynamic change of their morphology. As discussed,

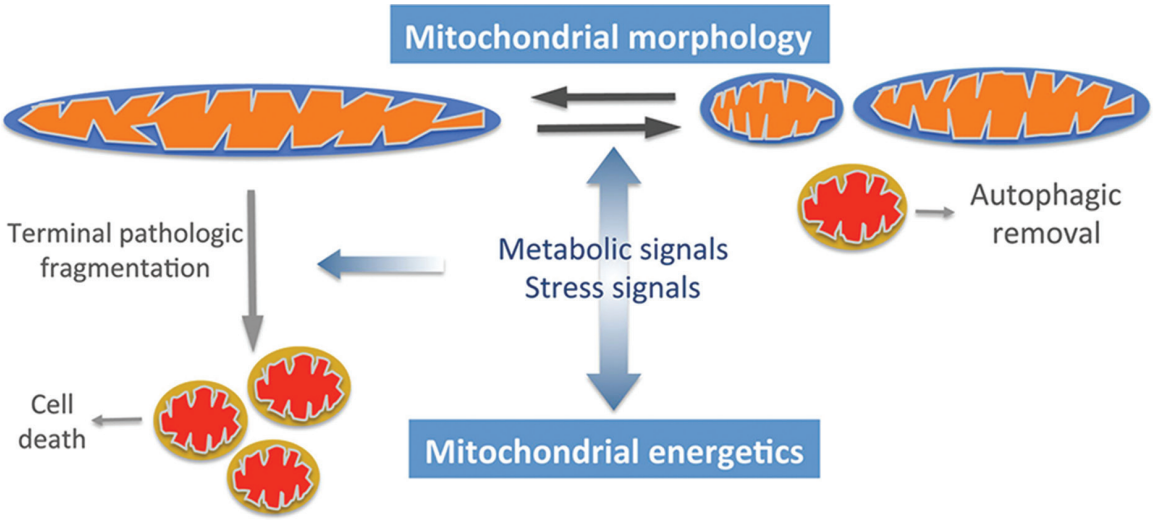

Figure 3. Mitochondrial morphology and function correlation. Mitochondrial morphology is in equilibrium at steady state through balanced fission and fusion. Fission and fusion in normal conditions maintains healthy mitochondrial population by separating and eliminating dysfunctional mitochondria through autophagy. Changes in environment such as nutritional switch or stress shift the fission-fusion balance and alter mitochondrial morphology as well as bioenergetic states. This correlation is bidirectional, as changing mitochondrial morphology alters bioenergetic state and the converse is also true. When metabolic insult and stress is too much for mitochondria to handle, mitochondrial morphology becomes terminally altered and cell death ensues.

cells change mitochondrial shape in response to external stresses or stimulations by regulating fission and fusion through cellular signaling pathways (Figure 3). By altering mitochondrial morphology, cells may be able to balance local as well as overall cellular energy need by optimizing their respiratory function. In this context, how the external morphological change affects bioenergetic activity of mitochondria would be a next question to address. Control of respiration coupling by the morphological change observed with altered fission is an attractive idea; however, whether it can be a unifying mechanism applicable for defining the form-function correlation in other conditions needs further evaluations. Hackenbrock's original notion that mitochondrial internal structure changes dynamically in different respiratory states raises new exciting questions in relation to morphological regulation of mitochondrial bioenergetics. Does overall external shape change through mitochondrial fission and fusion occur in coordination with internal organization dynamics in changing metabolic conditions? If so, which one occurs first, external or internal changes, and what coordinates it? It is possible that mitochondrial fission/fusion proteins may play an additional/alternative role in regulating internal mitochondrial structure. Indeed, as reported recently, the fusion protein OPA1 has been shown to regulate cristae structure and respiratory complex assembly (75). Whether this activity of OPA1 operates in conjunction with mitochondrial fusion remains to be tested. Addressing these questions will greatly expand our understanding of the mechanisms and pathophysiological significance of mitochondrial shape change in changing metabolic conditions.

6. ACKNOWLEDGMENTS

We thank Dawn O'Brien for critical reading. This work was supported by National Institutes of Health Grants DK061991 and DK078618 to YY.

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Key Words: Mitochondria, Fission, Fusion, Morphology, Dynamics, DLP1, Drp1, Mfn, OPA1, Bioenergetics, Review

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