

FORUM REVIEW ARTICLE

Mitochondrial Morphology in Metabolic Diseases

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Abstract

Significance: Mitochondria are the cellular energy-producing organelles and are at the crossroad of determining cell life and death. As such, the function of mitochondria has been intensely studied in metabolic disorders, including diabetes and associated maladies commonly grouped under all-inclusive pathological condition of metabolic syndrome. More recently, the altered metabolic profiles and function of mitochondria in these ailments have been correlated with their aberrant morphologies. This review describes an overview of mitochondrial fission and fusion machineries, and discusses implications of mitochondrial morphology and function in these metabolic maladies. **Recent Advances:** Mitochondria undergo frequent morphological changes, altering the mitochondrial network organization in response to environmental cues, termed mitochondrial dynamics. Mitochondrial fission and fusion mediate morphological plasticity of mitochondria and are controlled by membrane-remodeling mechanochemical enzymes and accessory proteins. Growing evidence suggests that mitochondrial dynamics play an important role in diabetes establishment and progression as well as associated ailments, including, but not limited to, metabolism–secretion coupling in the pancreas, nonalcoholic fatty liver disease progression, and diabetic cardiomyopathy. **Critical Issues:** While mitochondrial dynamics are intimately associated with mitochondrial bioenergetics, their cause-and-effect correlation remains undefined in metabolic diseases. **Future Directions:** The involvement of mitochondrial dynamics in metabolic diseases is in its relatively early stages. Elucidating the role of mitochondrial dynamics in pathological metabolic conditions will aid in defining the intricate form–function correlation of mitochondria in metabolic pathologies and should provide not only important clues to metabolic disease progression, but also new therapeutic targets. *Antioxid. Redox Signal.* 19, 415–430.

Introduction

MITOCHONDRIA ARE HIGHLY dynamic organelles constantly changing shape and location in the cell (6, 7). Mitochondrial shapes vary from small spherical and oval, typical textbook-depicted mitochondria, to interconnected filamentous networks. Small spherical mitochondria represent a fragmented or profission state, whereas the interconnected networks are representative of a profusion phenotype. The nature of these networks changes in response to environmental cues and varies greatly even within similar cellular lineages in the absence of stressed conditions (Fig. 1). The identification of core machinery responsible for the mechanical scission or fusion of mitochondria has allowed a rapid advance of this research field, but new mechanistic details are ever evolving through discoveries of additional players in these fundamental cellular processes. The necessity of mitochondrial dynamics is apparent by the requirement of all four mechanoenzymes in embryonic development (24, 35, 70, 138).

In cell systems, their knockdown is tolerable to an extent; however, bioenergetic and cell survival deficiencies are evident. Production of adenosine triphosphate (ATP) through oxidative phosphorylation in the electron transport chain (ETC) complexes localized within the inner membrane of mitochondria is a primary ascribed function of mitochondria. Dysregulation of this highly regulated process results in bioenergetic deficiencies and/or production of damaging reactive oxygen species (ROS) contributing to organ damage. These maladies have been described in the development of metabolic pathologies.

The implication of mitochondrial dynamics in metabolic diseases is a relatively new concept. Given the energetic implications of these pathologies, mitochondrial involvement is obligate. The nature of changes associated with bioenergetic fluctuations and the role mitochondrial dynamic plays or responds to are an interesting new frontier in the deconvolution of the mechanisms underlying these diseases. Metabolic diseases are, at their root, a problem of excess, specifically in an

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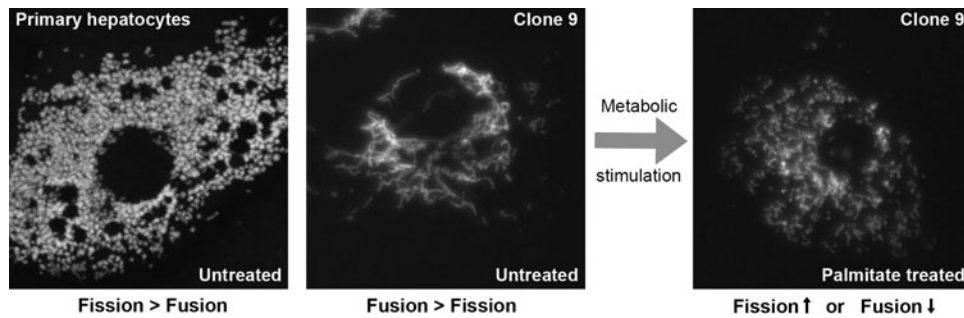


FIG. 1. Mitochondrial network morphology is varied between similar cell types of similar lineage and under metabolic stress. (Left panel) Primary mouse hepatocytes, under unstressed culture conditions, display a small, spherical fragmented mitochondrial morphology filling the cytoplasm, suggesting that mitochondrial fission is favored over fusion. The mitochondrial network was visualized by fluorescence microscopy after infection with adenovirus expression of mitochondria-targeted GFP (mtGFP). Image courtesy of Dr. Hakjoo Lee. (Middle panel) Mitochondria of immortalized Clone 9 hepatocytes cultured in unstressed conditions, stably expressing mtGFP, are observed as a more perinuclear interconnected network with longer tubular structures extending out toward the periphery of the cell, indicating that mitochondrial fusion is favored over fission in these cells. (Right panel) Treatment of mtGFP Clone 9 hepatic cells with 250 μ M palmitate resulted in the rapid fragmentation of mitochondria. The fragmented mitochondrial morphology can be accomplished either through the increase in the frequency of fission events or through the suppression of fusion events.

insulin-resistant state, where both hyperglycemia and hyperlipidemia are present. The progression of these pathologies is dependent upon how mitochondria handle this excess. Hence, how mitochondrial dynamics govern this process is an intriguing question. We previously reviewed the implication of mitochondrial dynamics in diabetes (145), and the current review extends that discussion to commonly associated diabetic complications: nonalcoholic fatty liver disease (NAFLD) and diabetic cardiomyopathy (DCM). We will first briefly summarize mitochondrial fission and fusion machineries and further discuss recently discovered proteins implicated in these processes. We will then discuss the current understanding of mitochondrial dynamics in the context of metabolic diseases commonly associated with diabetes and insulin resistance, namely NAFLD and DCM.

The Core Machinery for Mitochondrial Fission and Fusion

Mitochondrial fission and fusion require membrane remodeling mediated by dynamin-related GTPases. In mitochondrial fission, dynamin-like/related protein 1 (DLP1/Drp1) forms oligomeric ring structures around the mitochondrial membrane, constricting and severing it in a GTPase-dependent manner (126, 148). The fused mitochondrial network observed with expression of dominant-negative (DN) DLP1 mutants or DLP1 knockdown/knockout supports its necessity in the fission process (54, 70, 81, 126). The requirement for DLP1's GTPase activity and its assembly into oligomeric complexes is supported by the DN effect of GTPase- and middle-domain mutants (22, 108, 126). Recently, cryoelectron microscopy (EM) was used to visualize the spiral ring structure of Dnm1, the yeast homolog to DLP1, and its constriction upon addition of and hydrolysis of guanosine triphosphate (GTP) (89). Structurally, DLP1 contains a middle domain, an unconserved domain, and a C-terminal coiled-coiled (CC) domain following the N-terminal GTPase domain (Fig. 2). The middle domain has been implicated in the oligomerization of DLP1 required for its membrane-constricting

properties (22), and the CC domain interacts with the N-terminal domains to regulate the GTPase activity. These domains of the protein are the subject of multiple post-translational modifications, including phosphorylation (21, 31, 61, 132, 151), S-nitrosylation (25), SUMOylation (11, 51, 62, 139), and ubiquitylation (94, 144), affecting DLP1 function, interactions, stability, and subcellular localization.

DLP1, unlike the other GTPases of mitochondrial dynamics, lacks a transmembrane domain and is therefore not restricted to the mitochondrial outer membrane. DLP1 is localized diffusely in the cytosol, with only a small portion localized to mitochondria (124, 126, 147), where it forms concentrated foci observed by immunofluorescence (107, 126). The punctate localization of DLP1 in foci at the mitochondrial membrane has been ascribed to the presence of a receptor in the outer mitochondrial membrane (OMM). Until recently, the role of the mitochondrial DLP1 receptor was thought to be occupied only by hFis1, a small helix-rich protein anchored in the OMM (129, 146) (Fig. 2). A recent study by Otera *et al.* suggested the mitochondrial fission factor (Mff) (Fig. 2) as the OMM receptor for DLP1 (103). Mff was originally identified as an OMM-bound protein with similar requirements in mitochondrial fission as hFis1 (56). Overexpression of hFis1 fragments mitochondria by increasing fission, which requires DLP1 function (129, 146). Similarly, the overexpression of Mff induced mitochondrial fragmentation in HeLa cells (103). Otera *et al.* indicated that the Mff-mediated mitochondrial fragmentation occurs in the absence of hFis1, suggesting that the hFis1 function is dispensable in the presence of Mff. Both hFis1 and Mff contain C-terminal transmembrane domains that allow their functional N-terminal regions exposed to the cytosol (56, 103, 129, 146). The cytosolic domain of hFis1 contains six α -helices of which the core four helices, α 2– α 5, form two tandem tetratricopeptide repeat (TPR) motifs that are known to participate in protein–protein interactions (40, 130). DLP1 was shown to interact transiently with the hFis1 TPR, which is regulated by the N-terminal first α -helix of hFis1 (120, 150) (Fig. 2). Mff interacts with DLP1 in a transient nature as well, requiring crosslinking to detect the

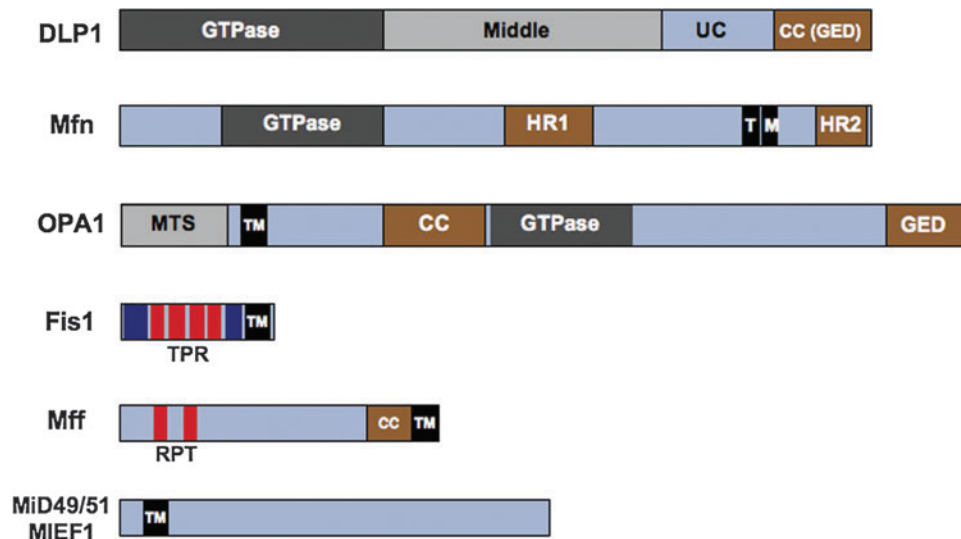


FIG. 2. Characterized domains of the core mitochondrial dynamics machinery. Dynamin-like protein 1 (DLP1), mitofusin (Mfn), and optic atrophy 1 (OPA1) are GTPases. DLP1 contains an N-terminal GTPase domain conserved with the dynamin family of proteins, a middle domain involved in higher-order self-assembly of DLP1, an unconserved domain (UC), and a coiled-coil (CC), or a GTPase effector domain (GED), functioning in protein-protein interactions. Mfn contains two transmembrane domains (TM) that cross the outer mitochondrial membrane (OMM) twice, which leaves the GTPase domain and heptad repeats 1 and 2 (HR1 and HR2) exposed to the cytosol. OPA1 contains a mitochondrial-targeting signal (MTS), which targets the protein to the inner mitochondrial membrane. The C-terminal portion of the protein containing the GTPase, CC and GED remains exposed to the intermitochondrial space. Fission protein 1 (Fis1), mitochondrial fission factor (Mff), and mitochondrial elongation factor 1 (MIEF1/MiD51) are the DLP1 receptor proteins. All contain a transmembrane domain anchoring them to the OMM. No other domains of the MIEF1/MiD51 are described; however, the C-terminus was required for DLP1 interaction. Mff contains a cytosolic CC domain and two short repeats (RPT) conserved in metazoans and implicated in DLP1 interaction. Fis1 contains six α -helices in its cytosolic face with the four internal helices forming a tetrapeptide repeat (TPR) involved in DLP1 binding. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

interaction by immunoprecipitation (56, 103). Although this interaction is transient, coimmunoprecipitation studies in combination with hFis1 demonstrated that interaction between DLP1 and Mff was preserved in more stringent conditions than that of DLP1 with hFis1 (103). The authors suggest that the interaction requires the two conserved N-terminal repeats within the first 50 amino acid residues, as deletion of the first 50 N-terminal residues resulted in a reduction of DLP1 at the mitochondria and in mitochondrial fission (103). hFis1 has been shown to localize evenly throughout the mitochondrial surface (71, 130), and changes of hFis1 levels did not affect the DLP1 distribution/level in mitochondria (81, 130). Conversely, Mff distributed along the mitochondria in punctate clusters, colocalizing with DLP1 by immunofluorescence (103). Ribonucleic acid interference (RNAi) knockdown of Mff eliminated punctate DLP1 localization at the mitochondria, suggesting that Mff was required for DLP1 recruitment. Strengthening this argument, when Mff was mistargeted to the plasma membrane, DLP1 redistributed to the plasma membrane where they co-localized (103). These studies support a role for Mff as a receptor for DLP1 at the mitochondrial surface, but do not exclude a role for hFis1 in the mechanism of mitochondrial fission outside of targeting of DLP1 to the mitochondria. It is likely that other factors, including signal-mediated modifications in participating proteins and their relative ratios, may play a role in regulating DLP1-mediated mitochondrial fission.

Mitochondrial fusion requires sequential fusion of outer and inner membranes. Two isoforms of the dynamin-related GTPase mitofusin (Mfn) 1 and 2 exist in mammals and mediate the OMM fusion (113–115). Anchored to the OMM, their topology is unique, crossing the OMM twice, which results in a small loop within the intermembrane space leaving the GTPase domain and the two heptad-repeat (HR) domains, HR1 and HR2, in the cytosol (115). Mfn1 molecules can interact intermitochondrially to tether adjacent mitochondria together through their HR2 domains, promoting mitochondrial fusion (79). Recently, it has been shown that the HR1 domain of Mfn2 interacts with HR2 and also with the CC domain of the fission protein DLP1 (67). The interaction between HR1 and HR2 exerted an inhibitory effect on fusion, while interaction of the HR1 domain with the DLP1 CC domain appeared to promote fusion. Additionally, Mfn2 has recently also been described to serve the function of tethering mitochondria to the endoplasmic reticulum, allowing Ca^{2+} communication between the organelles (38). This newly ascribed function may explain the observation of enhanced levels of Mfn2 expression in skeletal and cardiac tissue (114), where Ca^{2+} transients are crucial to muscle motive force. In combination with Mfn1, optic atrophy 1 (OPA1) is required to complete mitochondrial fusion by mediating fusion of the inner mitochondrial membrane (IMM) (27). OPA1 is anchored in the IMM by its N-terminus with the bulk of the molecule facing the intermembrane space. This brings the loop

structure of the Mfn proteins in proximity to the C-terminus of OPA1, which would allow coordinate communication in the completion of mitochondrial fusion (27, 35, 59, 99). OPA1 is extensively modified at both the transcriptional and post-translational levels, expressing eight different forms in humans through alternative splicing (39) that are further processed by multiple proteases, generating soluble forms of OPA1 (28, 44, 46, 58, 63, 69, 127). This processing gives rise to long and short forms of OPA1, both required for proper IMM fusion (127). In addition to its function in mitochondrial fusion, cleavage of OPA1 by the presenilin-associated, rhomboid-like (PARL) protease produces a soluble form of OPA1 in the intermembrane space, where it forms a heteromeric complex with the long membrane-bound form at the neck of the cristae to keep the cristae junction closed, preventing cytochrome *c* release (28, 55).

Mff and Mitochondrial Dynamics Protein 49/51/ Mitochondrial Elongation Factor 1, New Proteins in Mitochondrial Fission/Fusion

While DLP1, hFis1, Mfn, and OPA1 are the most studied proteins for mitochondrial fission and fusion, additional proteins have been identified and implicated in mitochondrial dynamics. For the purpose of this review, proteins identified to be physically and/or functionally interactive with mitochondrial fission and fusion are summarized in a table (Table 1). The mechanisms of mitochondrial fission and fusion were initially studied in yeast, and there are still several gaps exist with mammalian systems. Yeast Ugo1 participates in mitochondrial fusion, functioning as a linker protein between Fzo1 (Mfn homolog) and Mgm1 (OPA1 homolog) (121). In mammals, however, although the required communication

TABLE 1. LOCATION AND FUNCTION OF PROTEINS IMPLICATED IN MITOCHONDRIAL FISSION AND FUSION

Component	Local	Function	Altered expression	Mitochondrial phenotype
DLP1/Drp1	Cyt OMM	Fission (mitochondrial membrane severing)	OX KD/KO	None or elongation (67, 108, 126) or enhanced fragmentation (131) Elongated mitochondria (70, 81)
Fis1	OMM	Fission (possible receptor for DLP1 or other factors)	OX KD	Extensive fission (71, 129, 146) Extensive fusion (129)
Mfn1	OMM	Outer membrane fusion (mitochondrial tethering)	OX KD/KO	Clustered, fragmented mitochondria (114, 115) Increased fission (24, 49, 79)
Mfn2	OMM ER	Outer membrane fusion; ER-mitochondria tethering	OX KD/KO	Clusters of fragmented mitochondria (68, 115) Enhanced fragmentation (24)
OPA1	IMM IMS	Inner membrane fusion; crista junction regulation	OX KD/KO	Mitochondrial elongation (27) Fragmentation, vacuolar crista (99)
MTP18	IMM	Fission regulation	OX KD	Fragmented mitochondria (134) Interconnected mitochondria (134, 136)
Endophilin B1	Cyt	Fission (membrane curvature)	KD	OMM dissociation from IMM (75)
GDAP1	OMM	Fission promotion	OX KD/KO	Extensive fission (97) Mitochondrial elongation (32)
Mff	OMM	Fission promotion; DLP1 receptor	OX KD/KO	Extensive fission (103), none (56) Mitochondrial elongation (56, 103)
MARCH-V	OMM	Ubiquitin E3 ligase modifying DLP1/Fis1/Mfn2	KD	Fragmented mitochondria (94, 144) or elongation (76)
MitoPLD	OMM	Fusion promotion	KD	Fragmented mitochondria (26)
MIB	Cyt Mito	Negative regulator of Mfn	OX KD	Fragmented mitochondria (49) Elongated mitochondria (49)
SLP-2	IMM	Stress-induced fusion	KD	None (135)
MIEF1/MiD51	OMM	Fission decrease; DLP1 receptor	OX KD	Elongated mitochondria (104, 158) Fragmented mitochondria (158) and elongated (104)

Cyt, cytosol; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; ER, endoplasmic reticulum; IMS, intermembrane space; OX, overexpression; KD, knockdown; KO, knockout; DLP1, dynamin-like protein 1; Drp1, dynamin-related protein 1; Fis1, fission protein 1; OPA1, optic atrophy 1; MTP18, mitochondrial protein 18 kDa; GDAP1, ganglioside-induced differentiation-associated protein 1; Mff, mitochondrial fission factor; Mfn, mitofusin; MARCH-V, membrane-associated RING-CH-V; MitoPLD, mitochondrial phospholipase D; MIB, Mfn-binding protein; SLP-2, stomatin-like protein 2; MIEF1, mitochondrial elongation factor 1.

between Mfn and OPA1 in completing fusion of both membranes suggests its existence (27, 35, 59, 99), a mammalian Ugo1 homolog remains to be identified. The mitochondrial fission process in yeast requires an adaptor protein, Caf4 or Mdv1, that links Dnm1 to Fis1 during fission (57, 133, 155). However, homologs for these adaptor proteins have remained elusive in mammalian systems. Recent reports describing new components restricted to animal cells suggest that mitochondrial fission (and fusion as well) might have evolved differently in yeast and metazoan, which may help translating our understanding of the mitochondrial fission/fusion process from yeast to mammalian systems. As mentioned above, the recent report by Otera *et al.* has promoted Mff as a more-likely mammalian mitochondrial receptor for DLP1 (103). This report and that of Gandre-Babbe and van der Bliek, in their discovery of Mff (56), observed a consistent requirement for Mff in mitochondrial fission where knockdown of the protein resulted in extensive elongation of the mitochondrial network. However, the two reports contradict with each other in the ability to enhance mitochondrial fission by overexpression and to inhibit mitochondrial profusion phenotypes. Gandre-Babbe and van der Bliek showed that overexpression of Mff did not induce mitochondrial fragmentation, an effect similar to DLP1 overexpression, suggesting that unlike hFis1, Mff and DLP1 are not the rate-limiting factors in mitochondrial fission. In contrast, Otera *et al.* indicated robust fragmentation of mitochondria upon Mff overexpression. Additionally, Otera *et al.* reported that Mff was required for OPA1 knockdown-induced fission, whereas Gandre-Babbe and van der Bliek observed that Mff knockdown only partially recovered from mitochondrial fragmentation induced by Mfn DN mutants (56, 103). The reports also differ in the requirement for hFis1 in the induction of apoptosis under cellular insult. While Otera *et al.* found it dispensable, Gandre-Babbe found both Mff and hFis1 to have similar requirements, but the stimuli used to promote cell death varied (56, 103). This raises the possibility that the recognition and induction of apoptosis may be insult specific. In support of this, Gandre-Babbe and van der Bliek reported that although both hFis1 and Mff interact, they were found in similar-sized, but separate, complexes with DLP1 (56). Although it is clear that Mff, hFis1, and DLP1 are *bona fide* components in mitochondrial fission, further investigation is necessary for defining their functional interactions.

Additional mitochondrial membrane-bound proteins, mitochondrial dynamics protein 49 and 51 (MiD49 and MiD51)/mitochondrial elongation factor 1 (MIEF1), were independently identified by two groups (104, 158). The proteins MiD49 and 51 were discovered through a screen of uncharacterized human genes for their ability to alter mitochondrial morphology (104), whereas MIEF1 (MiD51) was identified from a screen of a green fluorescent protein (GFP)-tagged library by virtue of its mitochondrial localization (158). MiD49 and MiD51/MIEF1 share 45% homology and a highly conserved N-terminal transmembrane domain that anchors them to the OMM (104, 158). All studies carried out by Zhao *et al.* utilized only the protein identified as MiD51 or their nomenclature MIEF1. Zhao *et al.* showed that MIEF1 knockdown induced mitochondrial fragmentation (158). In contradiction to this, Palmer *et al.* showed that knockdown of both MiD49 and 51 by RNAi resulted in fused mitochondria (104). Conclusions drawn from these results may be difficult to in-

terpret due to the variable nature of knockdown by RNAi. Zhao *et al.* knocked down MIEF1/MiD51 alone, inducing mitochondrial fragmentation, whereas Palmer *et al.* did both MiD49 and MiD51 simultaneously and observed elongated mitochondrial morphology. These opposite results led to differing proposed roles for the newly discovered proteins as profission by Palmer *et al.*, and conversely profusion by Zhao *et al.* Despite this discrepancy, both reports demonstrated that overexpression of MiD49 or MiD51/MIEF1 resulted in a profusion phenotype with elongated mitochondrial tubules collapsing into a cluster of tubules in a time-dependent manner (104, 158). Counter intuitively, overexpression also resulted in the enhanced mitochondrial association of the fission protein DLP1, which would predict the profission phenotype. Palmer *et al.* suggest that this elongated mitochondrial morphology may be an artifact, arising from diffuse mislocalization of overexpressed MiD49/51 at the mitochondrial surface, which prevents the formation of fission foci. Nevertheless, MIEF1 interacts with DLP1 as judged by immunoprecipitation and yeast two-hybrid, and this interaction was independent of hFis1, Mff, and Mfn2. Recruitment of DLP1 did not require DLP1's GTPase activity, recruiting the GTPase-defective DLP1-K38A mutant to the mitochondria. However, oligomerization-defective DLP1-A395D or G363D mutants did not localize to mitochondria upon MiD49/51 MIEF1 overexpression (104, 158). The definition of the role that MiD51/MIEF1 plays in DLP1 recruitment to the mitochondrial membrane has a strong similarity to that of Mff. Although they both appear to have a DLP1 receptor quality in that respect, their functional consequence appears to be in exact opposition: Mff promoting fission and MiD51/MIEF1 promoting fusion. MIEF1 was also competent to interact with both hFis1 and DLP1, and they were found to be contained in differing complexes with MIEF1 (158). Zhao *et al.* speculate that the relative amounts of hFis1 and MIEF1 could regulate the fate of DLP1 in its role in fission, either profission in complex with hFis1 or profusion in complex with MIEF1. In line with this speculation, a recent study indicated functional and physical interaction between Mfn2 and DLP1, which promotes mitochondrial fusion (67). DLP1 recruitment by MiD49/50/MIEF1 would decrease the profission activity of DLP1 by inhibiting GTP binding (158), which allows DLP1 available to interact with Mfn2 for profusion effect (Fig. 3). Given contradicting mitochondrial phenotypes reported by different groups that studied the same protein, further clarification is necessary to define how mitochondrial fission and fusion are regulated through Mff and MiD49/51/MIEF1. Aforementioned, it is of note that all three of these newly described proteins are not present in yeast (56, 103, 104, 158), and may represent evolutionary changes/advances in the regulation of fission and fusion. Mammals have turned out to contain additional factors and interactions in the fission/fusion machinery. This adds to the difficulty of deciphering the obligate interactions and regulation of mitochondrial fission and fusion in mammalian cells, which likely have additional levels of complexity.

Mitochondria in Metabolic Disease

Metabolic diseases are characterized by states of misregulated excess supply of energy to cells. Disruption of the body's normal energetic regulation, largely the result of insulin

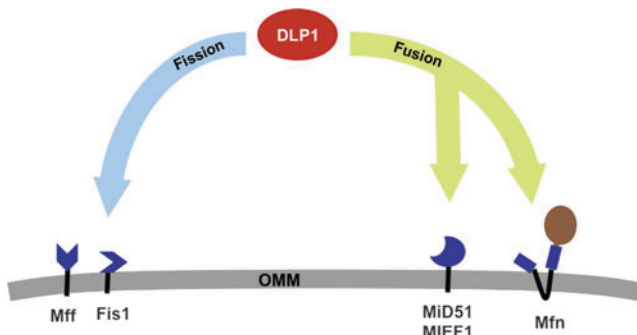


FIG. 3. A role of DLP1 interactions controlling the balance of mitochondrial fission and fusion through alternative complex formation. The identification of multiple new receptors at the mitochondrial outer membrane has suggested that enhanced recruitment of DLP1 to the OMM may not necessarily result in enhanced fission. Localized to the OMM, both hFis1 and Mff are profission receptors for DLP1. DLP1 interaction with hFis1 in mammalian systems is associated with enhanced mitochondrial fission. Recently, a second OMM receptor Mff was reported to serve a similar role in the absence of hFis1 protein. Reports differed slightly as to the ability of their overexpression to induce mitochondrial fragmentation and in their requirement to promote apoptosis. Analysis of complexes containing DLP1 at the mitochondria determined the two profission receptors to exist in distinct complexes, suggesting that their functional promotion of fission may occur through differing pathways. MIEF1/MiD51 promotes mitochondrial fusion when overexpressed. Enhanced MIEF1/MiD51 also increases DLP1 recruitment to the OMM while still promoting fusion. DLP1 interaction with MIEF1 alters its GTPase activity, possibly defining an underlying cause. The recent report that DLP1 interaction with Mfn2 supports mitochondrial fusion could also underlie this phenotype. Taken together, recruitment of DLP1 to MIEF1/MiD51 would result in its reduced GTPase activity, still allowing its interaction with Mfn and promoting fusion. While Mff, hFis1, MIEF1/MiD51, and Mfn are bound to the OMM, DLP1 retains the ability to traffic between the cytoplasm and the OMM. DLP1 recruitment to differing specific microdomains of these proteins then represents the regulatable process, likely coordinated by the extensive post-translational modification of DLP1, in directing fission or fusion at the OMM. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

resistance, causes enhanced demand on mitochondria to catabolize unrestricted influx of metabolites. The crossroads of mitochondrial bioenergetics and dynamics may serve to attempt to cope with this excess, but ultimately may contribute to the disease progression, as their abnormal morphology is observed in the pathology of these diseases. While our understanding of the role mitochondrial dynamics plays in these diseases is rudimentary, we will examine corollary changes in the form and function of mitochondria in a metabolic insult. The section will highlight our current understanding of morphological changes associated with metabolic diseases, NAFLD and DCM, commonly associated with diabetes, and observations of mitochondrial dysfunction associated with them.

Mitochondria in NAFLD

NAFLD is a progressive liver disease affecting 20%–30% of the population of western industrialized countries (5, 14, 29,

87). Advancement of the disease is hypothesized to occur through a two-hit mechanism where the first hit, steatosis, the accumulation of fat in hepatocytes, enables progression to nonalcoholic steatohepatitis (NASH) *via* either environmental or metabolic cues, termed second hits (36). This pathological condition is commonly accompanied by metabolic disorders prevalent in insulin resistance such as obesity, hyperglycemia, and dyslipidemia. In the context of NAFLD, the associated maladies create a feed-forward detrimental cycle in which insulin resistance in combination with excess adipose tissue releases triacylglycerol (TAG) from this tissue into circulation, a process normally suppressed by insulin through inhibition of hormone-sensitive lipase. Lipids are taken up into peripheral tissues or by the liver, where the lack of insulin sensitivity is also promoting the synthesis of fatty acids, adding to the lipid overabundance, which contributes to oxidative stress for the NASH progression as a second hit.

Mitochondria have been shown to play a major role in the development of oxidative stress in NAFLD through an increased production of ROS (106, 119). Mitochondria function in the catabolic processing of carbohydrates and fatty acids, through processing glycolysis end products and β -oxidation, to produce nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH₂) as input for the ETC. Under normal environmental conditions, this process is highly efficient or coupled with ATP production, occurring at a similar rate as O₂ consumption, the final acceptor of electrons through the ETC. Hyperglycemia and hyperlipidemia, stimuli reported to contribute to enhanced mitochondrial ROS production (15, 42, 95, 98, 118, 152), create an environment of metabolic excess with enhanced ETC inputs of NADH and FADH₂ at ETC complexes I and II, respectively. This could in turn cause inefficient electron transport, resulting in the production of mitochondrial ROS. In support of this, mitochondria have been observed to have a $\Delta\Psi_m$ threshold, above which ROS are produced from the ETC (8, 78). Increases in both β -oxidation (90, 116) and ROS production from mitochondria have been reported to play a role in the development of oxidative stress conditions in NAFLD (64, 106). Enhanced cellular ROS are thought to act in close proximity to their production and oxidize proteins, lipids, and DNA, thereby altering their ascribed function or rendering them nonfunctional. ROS produced at the mitochondria would then likely modify mitochondrial proteins, such as the ETC components, which may alter their function. In support of this notion, patients who have progressed from NAFLD to NASH displayed a reduced activity at all five ETC complexes (106). This suppressed function of the ETC leads to a predictable impaired production of ATP in NASH patients (30). Although the exact mechanism by which these ROS are produced is still the subject of investigation, it is clear that their production correlates with mitochondrial dysfunction observed in NAFLD.

Another prominent question regarding the pathological progression of NAFLD is whether steatosis leads to insulin resistance or vice versa. Studies in animal models disagree with respect to the causal factor. OLETF rats have a deficiency in their satiety signaling, causing hyperphagia and obesity (92), which manifest in the pathological condition of steatosis by 20 weeks of age (109, 110). Importantly, the emergence of this pathology is associated with mitochondrial dysfunction, with hepatic triglyceride accumulation preceding hepatic insulin resistance merely through the hyperphagic phenotype

(111). In this same model, a methionine–choline-feeding regimen resulted in hepatic steatosis, a condition that was aggravated by addition of high fat to the diet by promoting insulin resistance (102). The molecular mechanism by which free fatty acids (FFA), in this case palmitate, produce an insulin-resistant state, in an acute treatment, was determined to involve mitochondrial dysfunction and the production of ROS (95). Nakamura *et al.* demonstrated that mitochondrially derived ROS were responsible for insulin resistance through the activation of c-Jun N-terminal Kinase1/2 (JNK1/2) and subsequent suppression of insulin receptor substrate 2. The insulin-resistant phenotype was recovered by treatment with ROS-scrubbing compounds *N*-acetyl-L-cysteine and α -tocopherol, as well as inhibition of mitochondrial ETC complexes, or uncoupling with carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), indicated a causal role for ROS in activation of JNK.

Metabolic insult and mitochondrial dynamics

Morphological deformation of mitochondria has been observed in NAFLD, suggesting the involvement of mitochondrial dynamics in its pathological progression in patients (20, 116). Mitochondria were observed as round and swollen with the loss of discernable crista structure; instead, the mitochondria contained paracrystalline inclusion bodies. Sanyal *et al.* correlated the mitochondrial morphological defect with an environment of enhanced oxidative stress by demonstrating significant increases in nitrotyrosine in hepatocytes of patients with fatty liver disease and full-blown NASH (116). The underlying mechanism to these mitochondrial morphological changes remains to be defined, which will require the use of defined cellular systems to separate the complexity of the metabolic milieu *in vivo*.

Studies using *in vitro* cell systems have demonstrated a lipotoxic effect of high-fat incubation, particularly with the saturated fatty acid palmitate. Palmitate is the most prevalent saturated fatty acid in circulation, and its level is further elevated in patients with NASH (37). The lipotoxic effect has been observed in pancreatic β -cells (47, 93), cardiomyocytes (77, 101), and hepatic cell lines (50, 72, 84, 128, 140, 154). Molina *et al.*, recently described a role for mitochondrial dynamics in the progression of the lipotoxic effect, or lipid-induced apoptosis, of FFA incubation in pancreatic β -cells (91). This study used mitochondrial-targeted photoactivatable GFP (mtPAGFP) and demonstrated the dynamic nature of β -cell mitochondria. The mtPAGFP allowed observation of the spread of photoactivated GFP throughout the mitochondrial network and visualized dynamic mitochondria undergoing frequent fusion and fission events. Addition of a mixture of elevated glucose and palmitate to primary islet cells resulted in significant increases in mitochondrial fragmentation within 4 h of treatment, a phenotype not observed in elevated glucose alone. The altered mitochondrial morphology was completely reversed by the RNAi suppression of hFis1, which additionally eliminated the recruitment of DLP1 to the mitochondria and further suppressed the induction of apoptosis. From a physiological aspect, treatment with elevated glucose and fatty acids inhibited the secretion of insulin, and this was not recovered by hFis1 RNAi, suggesting that a morphological alteration of mitochondria, not the apoptotic process, may perturb the insulin secretion. Previous studies overexpressing

hFis1 reported mitochondrial fragmentation and promotion of apoptosis by increasing fission, a mechanism that was dependent on DLP1 function (129, 146). In contrast to this report, however, reduced levels of hFis1 did not prevent the localization of DLP1 to the mitochondrial membrane (81, 130). It cannot be ruled out that discrepancies may lie in the cell systems used, β -cells and HeLa cells. Given the recent reports of Mff (103), MiD49/50 (104), and MIEF1 (158) targeting DLP1 to the mitochondria, hFis1 may not serve as the *bona fide* receptor of DLP1, but rather works downstream in the fission process. This may help reconcile the observed delay in the induction of apoptosis with hFis1 and mitochondrial fission disruption under apoptotic stimuli (3, 12, 13, 54, 81), and its reported expendability in mitochondrial fission (103). Conceivably, the two receptor proteins Mff and hFis1 may function in varied capacities in the promotion of mitochondrial fission dependent upon environmental cues and normal network maintenance, but dissection of this will require further investigation. In the context of metabolic disease, enhanced levels of FFA alone are toxic to β -cells, leading to their apoptosis and subsequently reducing the available pool of insulin-secreting cells. Reduced levels of insulin in circulation could work in the feed-forward mechanism above, enhancing misregulated lipolysis and promoting lipid uptake in hepatic tissue.

In hepatic tissue or cell systems, reports are scarce regarding the mitochondrial morphology in enhanced abundance of FFA. It was shown that incubation of HepG2 cells and primary mouse hepatocytes with palmitate fragmented mitochondria (82, 154, 156). In the case of primary mouse hepatocytes, incubation with palmitate, but not unsaturated fatty acids, resulted in the loss of mitochondrial membrane potential, assessed by tetramethylrhodamine methyl ester fluorescence, and an observed release of cytochrome *c* into the cytoplasm (82). This was also accompanied by enhanced abundance of ROS. Human derived HepG2 cells displayed a similar fragmented profission phenotype 24 h after supplementation with palmitate (154, 156). Fragmented mitochondria corresponded to a significant decrease in the cellular ATP levels, in agreement with mouse hepatocyte studies where mitochondrial membrane potential was lost and enhanced ROS abundance was observed. Interestingly, a coordinate reduction in the abundance of Mfn2 at both transcript and protein levels was observed in the same time course (156). Simplistically, this suggests that the observed fragmented swollen mitochondria in patients with NASH could in fact be the result of decreased expression of the profusion GTPase Mfn2. In addition, the loss of the intramitochondrial structure was observed in the cristae in NASH patients (116), indicating a perturbation of the remodeling of the IMM. OPA1 is bound to the IMM and appears highly concentrated at the junction of cristae (59), giving structure to invaginations of the IMM, where the ETC complexes assemble. Reduced mitochondrial membrane potential or ATP levels following palmitate treatment would promote the increased proteolytic cleavage of OPA1 (43, 46, 63). Cleavage by OMA1 of the long-form OPA1 removes the inner membrane portion of OPA1 (63) and would release crista structure. The morphological change observed in patient samples is consistent with altered OPA1 abundance and/or processing. Additionally, processing of the long forms of OPA1 is consistent with the progression of apoptosis (3, 69), a reported consequence of studies using palmitate treatment

in HepG2 cells. Another consideration in the interpretation of the *in vitro* data is the time point in which these experiments were conducted, monitoring cells in 24 h after treatment. Insult by hyperglycemia has been shown to occur rapidly, causing mitochondrial fragmentation and increased ROS abundance within minutes of incubation with high glucose (152, 153). Furthermore, observations over a time course of 24 h demonstrated cyclic fragmentation and fusion that coordinate with ROS production (152), eventually causing cell injury in later time points (153). The reported *in vivo* observations show representative mitochondrial morphology of the full-blown disease (20, 116). To define whether mitochondrial morphological alterations contribute to NAFLD progression, these changes need to be examined during the progression of the pathological condition.

Previously, we defined a ROS production in metabolic excess, hyperglycemic conditions, and linked their production to a coordinate observation of mitochondrial fragmentation (152, 153). Inhibiting mitochondrial fission or increasing fusion normalized ROS levels in the high-glucose incubation (152). The ability to manipulate ROS levels through mitochondrial dynamics defined the morphological changes as upstream causal factor for the ROS production, implicating mitochondrial dynamics as a controlling factor regulating mitochondrial activity (152). While our inhibition of fission utilized the expression of DN-DLP1, another report showed that downregulation of hFis1 decreased the ROS level in acute high-glucose stimulation (137). We have observed a similar response in the suppression of palmitate-stimulated ROS production in a hepatic cell line with the overexpression of DN-DLP1 (unpublished data). The similar recovery of ROS insult in metabolically induced mitochondrial fragmentation, through excess glucose and/or palmitate, suggests a common role for mitochondrial fission in the progression in metabolic disease through oxidative stress while providing a potential therapeutic target for their treatment.

Mitochondria in DCM

Cardiac dysfunction is associated with a metabolic disease, and most commonly diabetes. Approximately 30% of diabetics develop heart disease (86). While atherosclerosis and coronary heart disease are accelerated by diabetes, DCM, characterized by decreased left ventricular function and resulting in heart failure, can arise independent of these pathologies (60, 65, 112). Dysregulation of Ca^{2+} handling and metabolic changes are observed early in the disease. Deficiencies in myocardial efficiency are also observed (66), where oxygen consumption does not correlate with ATP production, suggestive of an uncoupled mitochondrial phenotype. Similar to the development of NAFLD, this pathology is also correlated with accumulation of triglycerides or steatosis in the cardiac tissue (88). Given the intimate role that mitochondria play in calcium handling and cellular metabolism, their dysfunction has been a target in defining the DCM progression.

The underlying mechanism of mitochondrial dysfunction in DCM has yet to be elucidated; however, the involvement of mitochondrial ROS production has been suggested by observation of enhanced levels of ROS in cardiomyocytes of animal models of types 1 and 2 diabetes and their resulting damage in enhanced nitrosylation and lipid peroxidation of cellular components (10, 125, 142, 143). The clinical progres-

sion of diabetes and resulting pathological condition of DCM are associated with increased levels of cardiac ROS and related oxidative stress (143), reduced cardiac efficiency (66), and morphological alterations of mitochondria, appearing swollen, rounded with the loss of discernable cristae (112). More recently, patient samples from the right atrium of diabetic patients confirmed similar findings in humans (1). Anderson and colleagues determined that patient samples from diabetic subjects contained nearly twice the amount of triglyceride as nondiabetic patients, giving them a steatotic phenotype. Using permeabilized myocardial fibrils, they determined that the steatotic cardiomyocytes were deficient in respiration of fatty acid substrates independent of mitochondrial fatty acid transport (1). This suggests that the mitochondrial energetic deficiency lies within mitochondrial components and is not the result of restricted access of substrate. Respiration with glutamate as a substrate was also impaired in diabetic samples; however, the use of succinate demonstrated similar results in oxygen consumption, suggesting that complex II was functional. Importantly, although diabetic samples were deficient in respiration with their most utilized substrates, all substrates tested resulted in higher H_2O_2 emission, which was further amplified by the reduction in the H_2O_2 scavenger glutathione (1). As one would anticipate, this leads to increases in oxidative damage in enhanced protein nitrosylation and 4-hydroxynonenal adducts. In a follow-up study, the group observed an increased propensity for mPTP opening with Ca^{2+} stimulation and an increase in the abundance of active caspase 9 in diabetic cardiac tissue samples over that of nondiabetic patients (2). These studies were the first direct evidence that the oxidative damage in human DCM is the result of mitochondrial dysfunction, which enhanced the propensity of cardiomyocyte death and correlates the disease progression with results from animal models. While this study did not examine mitochondrial morphology in human DCM, work in animal models has observed correlations between altered mitochondrial morphology and dysfunction during DCM progression.

Various rodent models have been used to dissect the progression of DCM, including the type 1 diabetic OVE26 mice, Akita mice, and streptozotocin (STZ)-injected models, as well as the type 2 diabetic *Lep^{ob}* and *Lepr^{db}* models. In all models tested, except Akita mice where oxidative stress was not observed, cardiac and mitochondrial functions were decreased (9, 10, 16, 18, 19, 41, 53, 73, 80, 96, 122, 123), and these decreases were associated with increases in oxidative stress (10, 125, 142, 143). The OVE26 mouse model develops a type 1 diabetic phenotype through the overexpression of calmodulin, specifically in pancreatic β -cells, leading to damage and extremely low levels of insulin secretion (48). Transmission EM of cardiac tissue of OVE26 mice demonstrated an extreme mitochondrial morphology disruption accompanied by disruption of the myofibril structure (83, 122, 143). Interfibrillar mitochondria (IFM), normally aligned between myofibers, appeared fragmented and disordered. At a higher magnification, cardiac mitochondria were less electron dense with disordered cristae (143). Changes in mitochondrial and myofibril structure were reflected in defects in contractile function. Western blotting of cardiac extracts established an environment of increased oxidative stress with enhanced levels of malondialdehyde-modified protein, and isolated cardiomyocytes produced enhanced levels of ROS observed

as increased dichlorofluorescein fluorescence. The overexpression of a catalase transgene in these mice recovered the mitochondrial and myofibril structural defects in addition to the contractile defects (143), suggesting that the production of ROS is integral in the myocardial defects of this type 1 diabetic model. In a follow-up study, the Epstein Lab further implicated the role of mitochondrial ROS production in cardiac morphological and physiological defects by demonstrating that the mitochondrial-specific ROS-scrubbing enzyme manganese superoxide dismutase (MnSOD) recovered the OVE26 cardiac maladies (122). Akita mice, which become diabetic by β -cell death in response to misfolded proinsulin (149), display a similar cardiac mitochondrial defect by EM (19). Despite their abnormal cardiac pathology, physiologically, they demonstrate very subtle contractile defects and retain mitochondrial coupling while lacking enhanced production of ROS and the resulting oxidative stress (18). Subsequent studies utilizing succinate and glutamate as substrates determined that Akita mice do have deficiencies in respiration in cardiac tissue, which results in suppressed ATP production (19). This suggests that ROS production may not be required for the development of DCM; however, it may exacerbate the physiological impairment upon metabolic insults, as type 2 models of diabetes demonstrate more robust impairment of contractile function and energetic deficiencies (16, 85). Despite the absence of ROS, there is a consistent abnormal mitochondrial morphology in cardiac tissue from these type 1 diabetic genetic mouse models, which correlates with suppressed energy production.

The type 2 diabetic mouse models Lep^{ob} and $Lepr^{db}$ result from a hyperphagic phenotype promoted by either deletion of functional leptin (157) or the knockout of the long-form leptin receptor (23), respectively. Studies by Dong *et al.* monitored the mitochondrial morphology present in the Lep^{ob} cardiac tissue and observed a swollen phenotype with disordered cristae, often with vacuoles within the IMM (41). Additionally, mitochondria were reported to be shortened, suggestive of fragmentation, and again coupled with increases in ROS, assessed by dihydroethidium. Treatment of cardiomyocytes with leptin suppressed the observed increases in ROS, which is interesting in that leptin sensitizes cells to insulin treatment, and defective insulin signaling is proposed to facilitate DCM progression. It would be interesting to see whether the mitochondrial morphology correlates with cardiac recovery during leptin treatment. In these models, oxygen consumption is increased while cardiac efficiency is decreased. The enzymes associated with fatty acid oxidation are increased in these animal models (10, 16, 85). Recently, using proteomic approaches, the Hollander lab defined increases in the subsarcolemmal mitochondrial (SSM) population and decrease of IFM in $Lepr^{db}$ mice (33). These alterations appear sufficient to enhance the oxygen consumption through fatty acid oxidation and were mimicked by cardiac-specific overexpression of peroxisome proliferator-activated receptor α (PPAR α) (52). However, the enhanced oxygen consumption did not result in increased production of ATP, defining their decreased cardiac efficiency and subsequent deficiencies in cardiac function. A proposed mechanism for this is through the overexpression of uncoupling proteins (UCPs) that relieve the buildup of the proton gradient (17). Results from Boudina *et al.* support this theory, demonstrating mitochondrial uncoupling in the $Lepr^{db}$ heart occurs through increased UCP activity (10). Importantly,

ROS may play a crucial role in activating these proteins, and precedence for this is established in ROS activation of UCP3 in skeletal muscle (45). If in fact, ROS enhancement is dependent on morphological change of mitochondria, as we have previously observed (152), then this places mitochondrial dynamics upstream of the observed uncoupling-mediated energy depletion and cardiac dysfunction in DCM.

Aside from genetic models, chemical manipulation to ablate pancreatic β -cells has been used to induce a type 1 diabetic phenotype through STZ (34, 73, 96, 141). The Hollander lab examined DCM in this model 5 weeks after the development of elevated blood glucose levels (34). As anticipated, contractile function was impaired in the hearts of diabetic mice. In this study, the investigators made a distinction between subpopulations of mitochondria in cardiac tissue, separating the IFM from SSM for assessment of mitochondrial function. Using a unique light scatter approach to determine both the size and internal complexity of isolated IFM and SSM, Dabkowski and colleagues determined that IFM recovered from diabetic cardiac tissue were smaller in size and retained less internal complexity than wild-type controls (34). This was not observed in the SSM samples. The reduced size of IFM could be explained by an enhanced fission, whereas the loss of internal complexity is consistent with the findings from Akita and OVE26 genetic models (19, 143). Respiration analyses of ETC components of the augmented IFM revealed deficiencies in respiration from complexes I, II, and III. Inefficient respiration from IFM fits with the loss of contractile force, as IFM juxtaposed to the sarcomere supply ATP for motive force. Coordinates with these changes in diabetic cardiac IFM were enhanced ROS production and oxidative stress in the form of

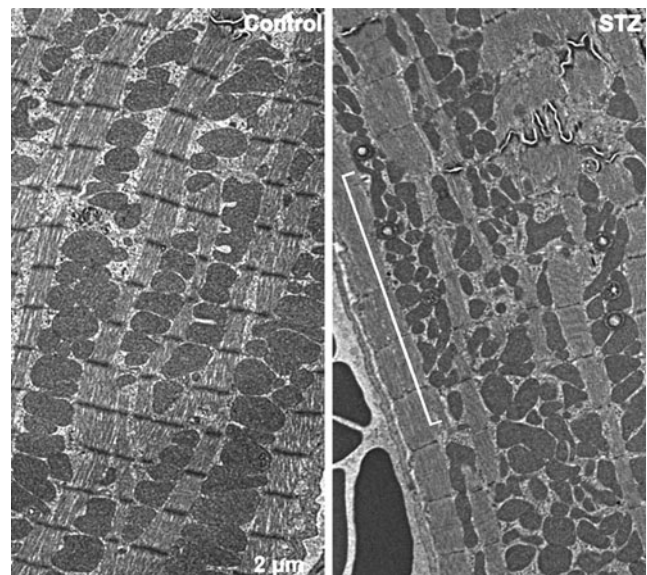


FIG. 4. Mitochondrial morphology in left ventricular samples from a type I diabetic mouse model is altered. Control heart shows normal tightly packed interfibrillar mitochondria (IFM) with normal uniform globular appearance. Most mitochondria are observed to span one sarcomere (*left panel*). Following 8 weeks of hyperglycemia, induced by streptozotocin (STZ) β -cell ablation, IFM are observed less uniform and more fragmented, highlighted by the bracketed region. The mitochondrial morphology is consistent with an increase in mitochondrial fission (*right panel*).

lipid modification (34). We have observed a similar fragmented phenotype of cardiac IFM with STZ-induced diabetes (Fig. 4), which correlated with enhanced ROS abundance (unpublished data). In the follow-up study by Williamson *et al.*, the same IFM were more susceptible to apoptotic cell death, with increased caspase activation, decreased mitochondrial membrane potential, and increased susceptibility to mPTP opening with enhanced proapoptotic protein expression (141). The smaller or fragmented characteristic of the IFM purports mitochondrial morphology as a pathological marker of DCM, as these mitochondria are more susceptible to mitochondrial apoptosis and subsequent tissue damage. Mitophagy, the autophagy of damaged mitochondria, has been reported to follow mitochondrial fission, selectively removing the depolarized daughter mitochondria (137). The accumulation of fragmented depolarized IFM in these studies could represent an improper control of mitophagy, possibly contributing to DCM. Alternatively, it may represent an inhibition of mitochondrial fusion, which would not allow the proper mixing of mitochondrial components. As discussed above in the context of NAFLD, while enhanced abundance of FFA can induce mitochondrial fission supporting the fragmented phenotype, whether this is the result of increased fission or decreased fusion will require further investigation (154, 156). It will be informative to examine changes in the abundance of fission and fusion factors in DCM, as studied in the *in vitro* palmitate treatment of HepG2 cells (156).

The suggestion of dynamic mitochondria seems to contradict a long-standing paradigm in the mitochondrial field that cardiac mitochondria were relatively static as far as morphological changes. *In vivo* observations support a presence of mitochondrial morphological change, but the question remains as to whether these are merely a consequence or causal to the disease progression. In addition to DCM, dysregulation of mitochondrial morphology has also been observed in dilated cardiomyopathy (117) and myocardial hibernation (74). Recently, a mutagenic screen described the first instance of a mitochondrial-remodeling protein defect, mutation of C452F in DLP1, resulting in cardiomyopathy (4). Interestingly, ischemia reperfusion injury (IRI) in cardiac tissue is also associated with mitochondrial fragmentation and can be circumvented by inhibiting mitochondrial fission with a small molecule, mdivi-1 (100). The study by Ong *et al.* implicates mitochondrial dynamics as a required process for cardiomyocyte damage in IRI and that may follow a scheme similar to the progression of DCM. The mechanism through which mitochondrial dynamics are altered in IRI remains unclear; however, mitochondrial fragmentation occurred upstream to apoptotic cell death placing misregulated mitochondrial dynamics as a possible causal factor in the injury. The overexpression of either the profusion GTPase Mfn2 or fission inhibitory DN-DLP1 delayed the progression of the apoptotic death in their HL-1 cell system (100). Treatment *in vivo* with a mitochondrial fission inhibitor, mdivi-1, was demonstrated to serve a protective role in IRI injury resulting in a smaller infarct size after the insult. Increased survival correlated with an observed increased abundance of elongated IFM, suggesting that elongated mitochondria are protective of IRI insult. In contrast to this, Papanicolaou *et al.* recently reported that cardiac-specific knockdown of the profusion GTPase Mfn2 conferred protective effects on mitochondria in adult cardiomyocytes under apoptosis-inducing conditions, delaying

mitochondrial permeability transition (105). Within the same study, however, the authors report that the suppression of Mfn2 in neonatal cardiomyocytes has opposing effects. Despite discrepancies in the functional outcome, mitochondrial dynamics and the proteins associated with these changes play roles in cardiac insult. The paradigm of immobile, restricted mitochondria in cardiac tissue has been replaced by the implication of mitochondrial dynamics in both the progression of cardiac disease, and its modulation as possible therapeutics to combat these diseases.

Perspectives

A wealth of evidence is present for a mitochondrial role in the progression of metabolic disease, but defining the nature of this role in human physiology has been difficult. Recent evidence has confirmed that the observed oxidative damage and mitochondrial dysfunction in animal models are also present in diabetic patients (1, 2). A prevailing commonality to metabolic disease pathologies is the suppression of ATP levels, which is the manifestation of mitochondrial bioenergetic deficiencies. Given the long-standing ascribed function of mitochondria as metabolic regulators of the cell, it is not

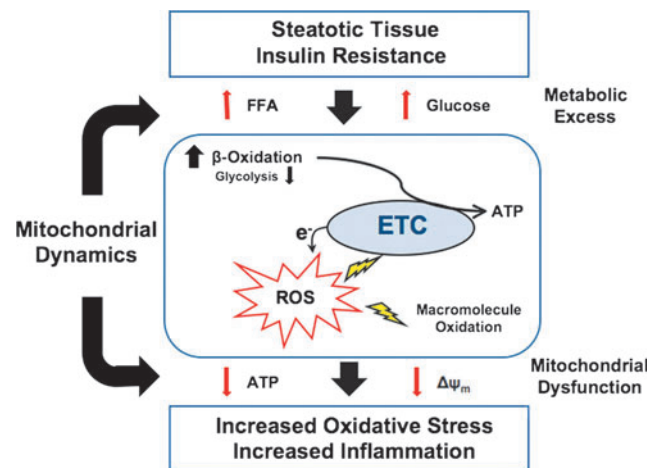


FIG. 5. A role of mitochondrial dynamics in the progression of metabolic disease. Nonalcoholic fatty liver disease (NAFLD) and diabetic cardiomyopathy both are associated with steatosis in their respective afflicted tissue type. This was hypothesized to be the first hit in NAFLD and is also associated with insulin resistance. Hyperglycemia and hyperlipidemia induce mitochondrial fragmentation, a process correlated with the enhanced production of mitochondrial reactive oxygen species (ROS). ROS modify cellular macromolecules, including proteins, lipids, and DNA, with functional consequences. Modification of electron transport chain (ETC) components in particular may exacerbate ROS production and bioenergetic deficiencies. These are reflected in the observed reduction of $\Delta\Psi_m$ and lowered adenosine triphosphate (ATP) levels despite an excess of ETC input. Suppression of these properties is also associated with processing of OPA1 with implications in cell viability and mitochondrial morphology. In this way, mitochondrial dynamics appear implicated in the characterized progression of metabolic disease associated with steatosis and may be integral to the hypothesized second hit in these conditions. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

surprising that they play an intricate role in metabolic diseases associated with hyperglycemia and hyperlipidemia.

Mitochondrial fission itself resulting from a hyperglycemic insult correlated with enhanced ROS production, but is ameliorated by suppression of fission. A similar mechanism could govern the dual insult of hyperglycemic and hyperlipidemic conditions in insulin-resistance-driven metabolic diseases (Fig. 5). Changes in the abundance of Mfn2 and OPA1 have been described to alter bioenergetic properties of mitochondria, with increased Mfn2 levels in increased energy demand and suppressed OPA1 levels associated with decreased respiration. While mitochondrial dynamics are intimately linked to mitochondrial energetics, their role causative or resultant remains an avenue of investigation. In either situation, changes in mitochondrial morphology implicate proteins involved in their remodeling in the etiology of metabolic disease. Their association with Bcl-2 proteins and the role of mitochondrial dynamics in apoptotic cell death also connects these proteins to the decision of cell life or death. Cyclically, this offers support for their role in maintaining or responding to energetic change. Defining the role of mitochondrial dynamics in pathological metabolic conditions will require more detailed assessments in the progression of the disease and correlations with mitochondrial bioenergetic and morphological properties. Mechanistically, defining the mitochondrial decision of fission or fusion from the perspective of functional interactions between implicated machineries will aid in defining the temporal execution in metabolic pathologies, such as NAFLD and DCM. While investigation of these disease states has been ongoing for decades, the involvement of mitochondrial dynamics is in its relatively juvenile stages and should yield important clues to their pathological progression and define new therapeutic targets.

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Abbreviations Used

ATP = adenosine triphosphate
CC = coiled-coil
CCCP = carbonyl cyanide <i>m</i> -chlorophenyl hydrazine
DCM = diabetic cardiomyopathy
DLP1 = dynamin-like protein 1
DN = dominant-negative
Drp1 = dynamin-related protein 1
EM = electron microscopy
ETC = electron transport chain
FADH ₂ = flavin adenine dinucleotide
FFA = free fatty acid
Fis1 = fission protein 1
GDAP1 = ganglioside-induced differentiation-associated protein 1
GFP = green fluorescent protein
GTP = guanosine triphosphate
HR = heptad repeat
IFM = interfibrillar mitochondria
IMM = inner mitochondrial membrane
IRI = ischemia reperfusion injury
JNK1/2 = c-Jun N-terminal Kinase1/2
MARCH-V = membrane-associated RING-CH-V
Mff = mitochondrial fission factor
Mfn = mitofusin
MIB = Mfn-binding protein
MiD49/51 = mitochondrial dynamics protein 49/51
MIEF1 = mitochondrial elongation factor 1
MitoPLD = mitochondrial phospholipase D
MnSOD = manganese superoxide dismutase
MTP18 = mitochondrial protein 18kDa
NADH = nicotinamide adenine dinucleotide
NAFLD = nonalcoholic fatty liver disease
NASH = nonalcoholic steatohepatitis
OMM = outer mitochondrial membrane
OPA1 = optic atrophy 1
PARL = presenilin-associated rhomboid-like
PPAR α = peroxisome proliferator-activated receptor α
RNAi = ribonucleic acid interference
ROS = reactive oxygen species
SLP-2 = stomatin-like protein 2
SSM = subsarcolemmal mitochondria
STZ = streptozotocin
TAG = triacylglycerol
TPR = tetratricopeptide repeat
UCP = uncoupling protein