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Regulation of Mitochondrial Morphology by Lipids

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Abstract

Although great progress has been made in identifying key protein factors that regulate mitochondrial morphology through mediating fission and fusion, signaling lipids are increasingly being recognized as important in the process as well. We review here roles that have been proposed for the signaling and bulk lipids cardiolipin, phosphatidic acid, lysophosphatidic acid, diacylglycerol, and phosphatidylethanolamine and the enzymes that generate or catabolize them in the regulation of mitochondrial morphology in yeast and mammals. Mutations in some of these enzymes are causal in a number of disease settings, highlighting the significance of controlling the lipid environment in this setting.

Keywords

mitochondria; fission; fusion; cardiolipin; phosphatidic acid

Introduction

Mitochondria have long been recognized for their role in cellular metabolism but are now appreciated to have other functions including regulation of intracellular Ca^{2+} homeostasis and apoptotic pathways (1). Once thought to be relatively static, mitochondria are now viewed as dynamic organelles that undergo fusion to form larger mitochondria and then divide via fission in response to changing demands for energy production, changes in cellular morphology, and cell division. Mitochondria have both an inner and an outer bilayer membrane, making the processes of fusion and fission more complicated than the fusion and fission events performed by other membrane organelles, and the mitochondrial events are thus mediated by specialized machinery. Mitochondrial fusion and fission are important in numerous settings such as cell proliferation, spermatogenesis, and neuronal function, and are linked to or affected in diseases such as Barth Syndrome (2–4), Parkinson's disease (5), and autosomal Dominant Optic Atrophy and Charcot-Marie-Tooth disease (6).

Mitochondrial fusion and fission are highly regulated processes, but the mechanisms through which they proceed are not fully understood. One area of investigation is focused on mitochondrial membrane lipids including cardiolipin (CL), phosphatidylethanolamine (PE),

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phosphatidic acid (PA), and diacylglycerol (DAG) (2), which are regulated in level by enzymes such as phospholipases, phosphatases, and acyl-transferases.

Several mechanisms have been proposed through which lipids such as these could influence mitochondrial morphology. One possibility is that the lipids might induce negative membrane curvature, which facilitates fusion and fission efficiency for single membrane organelles such as secretory vesicles fusing into the plasma membrane (7–10). Alternately, these lipids might recruit proteins that facilitate the fusion or fission process (11–13). Regardless of the mechanism, these signaling pathways appear to be important in the regulation of mitochondrial morphology (11, 12, 14, 15).

Cardiolipin recruits fusion and fission promoting proteins to mitochondria

Cardiolipin (CL), a negatively-charged lipid unique to mitochondria and an essential lipid for mitochondrial function, is a major component of the mitochondrial inner membrane where it comprises about 20% of the total lipid. CL is essential for fusion of the mitochondrial inner membrane through its interaction with the dynamin-related protein (DRP) Mgm1, a GTPase that facilitates fusion of the inner membrane (16, 17). Mgm1 is the yeast ortholog of mammalian Opa1, mutations in which cause autosomal Dominant Optic Atrophy (6).

Similar to Opa1, there are two isoforms of Mgm1, known as the long (l-Mgm1) and short (s-Mgm1) isoforms, which are inactive in their monomeric state but dimerize to form active GTPases that promote mitochondrial fusion. s-Mgm1 dimerization is CL-dependent and its GTPase activity increases in the presence of liposomes with lipid composition similar to that of the mitochondrial inner membrane, again in a CL-dependent manner (16). s-Mgm1 contains several positively-charged lysine residues in its protein sequence that are not critical for oligomerization but are critical for its interaction with negatively charged lipids such as CL, suggesting that the binding of s-Mgm1 to CL is itself critical for stimulation of s-Mgm1 GTPase activity (18).

While CL increases the GTPase activity of s-Mgm1, the same is not true for l-Mgm1 (16). Nonetheless, l-Mgm1 associates with liposomes in a CL-dependent manner. The direct association between s-Mgm1 and CL suggests that CL may function to target Mgm1 to the inner membrane. Although l-Mgm1 inserts into the inner membrane via its N-terminus, s-Mgm1 is located in the inter-membrane space and could therefore potentially reach either the inner or outer membrane (16). Thus, s-Mgm1's ability to bind CL as well as l-Mgm1's preference to insert into membranes containing CL highlights Mgm1's role as a CL-dependent inner-membrane fusion protein.

Mgm1 has also been proposed to interact with the mitochondrial outer membrane and to be essential for outer membrane fusion (19). Although CL content in the outer membrane is much lower than in the inner membrane, CL still represents 6% of the total lipids there (16) and can approach 25% of the lipids in contact sites (15), suggesting that there would be sufficient CL in the outer membrane to support Mgm1 activity there. Cardiolipin translocates in a regulatable manner from the inner membrane to the outer membrane, facilitating increased levels of the lipid there in settings such as apoptosis (20, 21).

CL may also interact with proteins that promote fission. α -Synuclein, which is associated with Parkinson's Disease, has been shown to promote mitochondrial fission in mammalian cells (22), and fragments artificial membranes in a CL-dependent manner, suggesting that CL is essential for the targeting of α -Synuclein to the mitochondrial membrane. Correlation between the fragmenting of mitochondria and neuronal degeneration potentially provides insight into the pathogenesis of Parkinson's Disease. This fission pathway functions in opposition to the fusogenic interaction between CL and Mgm1. On balance, however, CL appears to function more strongly as a fusogenic lipid, since CL deficiency results in fragmented mitochondria (17). Other proteins that are targeted to mitochondria through their interaction with CL include pro-apoptotic proteins, which may interact in the steady-state with CL found in mitochondrial contact sites in the outer membrane. Among these are tBid, which contains a three-helix domain that preferentially inserts into lipid membranes containing high levels of CL (23), and Bax (24), but tBid and Bax are unlikely to directly affect fusion or fission pathways.

Phosphatidylethanolamine deficiency fragments mitochondria

Another major lipid component of mitochondrial membranes is phosphatidylethanolamine (PE), which is found in significant concentrations in both the inner (25) and outer (26) membranes. In yeast, PE on the mitochondrial surface is primarily generated by the enzyme phosphatidylserine decarboxylase (Psd1); the mammalian ortholog is known as Psd (27). PE also plays important roles in mitochondrial fusion (27); yeast lacking Psd1 develop PE deficiency in mitochondrial membranes resulting in abnormal mitochondrial morphology characterized by extensive fragmentation and reduced ATP synthesis (25, 27). The PE-deficient mitochondria that do fuse are notable for incomplete mixing of the joined mitochondrial membranes, suggesting a role for PE in lipid mixing (27). Mgm1 activity is also decreased in Psd1 mutants, and the ratio between l-Mgm1 and s-Mgm1 is increased, which may be significant since mitochondrial fusion requires a careful balance between the two Mgm1 isoforms (27).

PE and CL may have overlapping roles in regulating mitochondrial morphology since combining Psd1 deficiency in yeast with the inability to make CL (i.e., in mutants for Crd1, a CL synthase) results in even more highly fragmented mitochondria and increased mortality (27). Combined PE and CL deficiency lowers the steady-state level of Mgm1 protein, potentially providing an underlying mechanism. Interestingly, in humans, Barth Syndrome is characterized by mutations in the enzyme tafazzin, which is a CL-remodeling enzyme; tafazzin dysfunction is associated with decreased concentrations of both PE and CL in membranes and highly fragmented mitochondria, suggesting that combined PE and CL deficiency may contribute to the pathogenesis of the disease (28).

Phosphatidic acid promotes mitochondrial aggregation and fusion

CL is also important for the production of phosphatidic acid (PA) at the mitochondrial surface through the action of MitoPLD, a mitochondrial-surface phospholipase D found in animals that cleaves CL to produce PA. Overexpression of MitoPLD in NIH3T3 cells decreases mitochondrial cardiolipin and increases phosphatidic acid on the mitochondrial

surface (12, 14, 15). However, MitoPLD can also function as an endonuclease in a different context and as such undertakes a critical role in piRNA biogenesis (29). MitoPLD is evolutionally most conserved with the PLD superfamily and bacterial endonuclease Nuc (15, 30), followed closely by the superfamily member yeast cardiolipin synthase (15). Although MitoPLD's hydrolytic activity on piRNA as an endonuclease is less technically challenging to demonstrate *in vitro* than its ability to hydrolyze cardiolipin to yield PA, the cardiolipin phospholipase activity and generation of PA is robust and easily observed *in vivo* with attendant effects on mitochondrial morphology.

PA can also be generated through other means such as by the conversion of lyso-PA (LPA) to PA by the enzyme LPA acetyltransferase (LPAAT), a step that occurs on peroxisome membranes during the fission process in yeast (11). PA is a negatively-charged and cone-shaped phospholipid, characteristics that allow PA to induce negative membrane curvature, thus making it generally important in membrane morphology (31). Evidence for PA's fusogenic role includes that mammalian cells overexpressing MitoPLD exhibit aggregated and enlarged mitochondria (15), a phenomenon seen with overexpression of Mfn1, another key fusion protein (32). Conversely, mammalian cells (15) or *Drosophila* (33) expressing a catalytically-inactive, dominant-negative MitoPLD allele or MitoPLD siRNA have fragmented mitochondria and reduced mitochondrial fusion, indicating that it is not the MitoPLD protein itself, but its product PA that is necessary for fusion. Supporting this hypothesis, enzymatic catabolism of PA on the mitochondrial surface by the phosphatidic acid-preferring phospholipase A₁ (PA-PLA₁), which cleaves PA to form LPA, or by the PA phosphatase Lipin 1b, which dephosphorylates PA to generate diacylglycerol (DAG), opposes the action of MitoPLD and promotes mitochondrial fragmentation in mammals (12, 14). Conversely, reducing levels of PA-PLA₁ or Lipin 1b results in mitochondrial elongation (12, 14). Interestingly, although PA-PLA₁ activity results in mitochondrial fission, mitochondrial glycerol-3-phosphate acyltransferase (Mt-GPAT), an enzyme that produces LPA through a different pathway via a non-PA substrate, is necessary for mitochondrial fusion in *C. elegans* and in HeLa cells (34), raising the possibility that LPA can also be fusogenic. This finding suggests that it could primarily be the decrease in PA concentration rather than the production of LPA that promotes fission in the setting of PA-PLA₁ activity. Alternately, Mt-GPAT is thought to localize to the interior of mitochondria (35) rather than to the surface where PA-PLA₁ functions, and lipids such as LPA and lysophosphatidylcholine (LPC) that facilitate membrane vesicle fusion and fission through effects on membrane curvature exert opposing effects depending on whether they are generated on the inwardly or outwardly bending sides of the membrane (36). Thus, LPA could have a pro-fission role when generated on the mitochondrial surface while having a pro-fusion effect when generated on the inner surface of the mitochondrial membrane.

While the mechanism through which PA affects fusion is unknown, in mammals it is likely to function in collaboration with Mfn1 and Mfn2, a pair of GTPases required for mitochondrial fusion (32). Mfn, a member of the dynamin superfamily of enzymes, is an integral outer membrane protein that trans-dimerizes to draw apposed mitochondria close together, bringing them within 16nm of each other and facilitating fusion via the action of the GTPase domain after multimerizing. In cells lacking both Mfn1 and Mfn2,

overexpression of MitoPLD no longer drives mitochondrial aggregation, suggesting that the action of Mfn to bring the mitochondria into close approximation is required for MitoPLD to function in trans to cleave CL to generate PA. In cells overexpressing MitoPLD, the mitochondria are apposed even closer to about 9nm apart, suggesting that PA generation may help drive the fusion process by bringing the outer membranes closer together than Mfn can achieve on its own (15).

PA has been implicated in other fusion-type processes, such as in SNARE-regulated exocytosis for both yeast and mammals (31), which may share some similarities with mitochondrial fusion. SNARE proteins associate with exocytic vesicles and the cell membrane, bringing the apposed membranes together in a manner somewhat analogous to how the Mfn proteins function. PA, in this setting, facilitates the fusion of the apposed membranes, both by enhancing the fusogenic properties of the SNARE proteins and by inducing membrane curvature, which lowers the activation energy barrier for the fusion event (7, 8).

PA can also play roles in fission both for membrane vesicles and mitochondria, due to its ability to recruit proteins that mediate vesicular membrane cleavage (37) or that generate lipids such as DAG that promote fission. In the latter case, MitoPLD-generated PA recruits the PA phosphatase Lipin 1b to the mitochondrial surface where it converts the PA to DAG, both inhibiting fusion through reduction in PA and stimulating fission (12). A C-terminal Lipin catalytic domain targets the mitochondrial fission site specifically, causing mitochondrial fragmentation in an activity-dependent manner.

Diacylglycerol promotes mitochondrial fission

Mammalian Lipin 1b contains a PA binding site in the middle of the protein that mediates its initial targeting to PA-rich mitochondrial surfaces, but also has a C-terminal catalytic domain that translocates to mitochondrial fission sites independent of PA (12), suggesting that it interacts there with a protein receptor. Taken together, these and other findings suggest that the Lipin 1b N-terminal domain sequesters the C-terminal fission-site interacting motif, as well as suppresses the activity of the catalytic site, and that a conformational change in the Lipin protein after initial recruitment by PA to the mitochondrial surface is necessary for access of the cryptic binding site to focus Lipin 1b onto fission sites and generate DAG there. The mechanism by which DAG facilitates fission is currently unknown.

Mitochondrial fission is often studied in the context of the dynamin-related protein Drp1 (or Dnm1, in yeast), a protein that mediates fission by constricting the mitochondrial membrane at fission sites (38). Drp1 recruitment is preceded by endoplasmic reticulum (ER) tubules first wrapping around and constricting the mitochondria at fission sites (39). Since Drp1 oligomerization forms a torus that is smaller than the circumference of typical mitochondrial tubules, it has been proposed that ER tubule constriction of mitochondria is necessary to reduce the mitochondrial diameter to a circumference that oligomerized Drp1 can span and then further contract and cleave. In mammalian cells, the ER constriction mechanism itself proceeds via the action of INF2, a protein that assists in actin polymerization (40), and

Myosin II (41). Both actin reorganization (42, 43) and myosin II activity (44, 45) are regulated by PA production and catabolism, suggesting a potential link between MitoPLD, PA-PLA₁, and Lipin 1b action, and actin/myosin II-regulated mitochondrial constriction.

DAG also regulates the actin cytoskeleton. One mechanism involves the recruitment of diacylglycerol kinase ζ , which converts DAG to PA and serves as a scaffolding protein to activate RhoA which directs actin cytoskeleton reorganization in many settings (46, 47). Taken together, a role for DAG in mitochondrial fission might be to promote actin filament polymerization at ER constriction sites in conjunction with INF2 and Myosin II, thus allowing the ER to constrict the mitochondrial fission sites to a circumference manageable for Drp1 activity.

DAG has already been implicated in the fission of mitochondrial-like double membrane-bound organelles, i.e. peroxisomes, in yeast. In this setting, PA is first produced at the future fission site and is then dephosphorylated to produce DAG, which recruits Vsp1, a GTPase that mediates fission, to the peroxisome membrane (11). However, it is unknown whether this mechanism involving DAG applies to mammalian peroxisome division as well. Another instance where DAG is involved in fission is in the Golgi complex, where, in mammals, it recruits Protein Kinase D (PKD) to the Golgi membrane, thereby leading to an increase in the concentrations of PA, DAG, and LPA, which then promote fission by inducing membrane curvature (48). The mechanisms by which DAG promotes fission in peroxisomes and in the Golgi complex may provide insight into the role of DAG in mitochondrial fission.

The Future of Research on the Role of Lipid Signals on Mitochondrial Morphology

While much is still unknown concerning the role of lipid signals in regulating mitochondrial morphology, lipids are clearly an integral part of the fusion and fission processes (Fig. 1). Given the important role of mitochondrial morphology in many cellular and physiological processes, research on the roles of lipids in fusion and fission may produce novel insights important in many disease settings. For example, decreasing mitochondrial fission through Drp1 inhibition has applications in preventing apoptosis in the contexts of kidney transplants and heart or brain ischemia reperfusion (49–52). Conversely, inhibiting mitochondrial fusion at the level of Mfn1 function is beneficial in increasing the tolerance of cardiomyocytes to stress-induced mitochondrial dysfunction (52, 53). Continued research on the mechanisms by which lipids interact with mitochondrial surface proteins that mediate fusion and fission may provide leads to develop new types of therapeutics.

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Figure 1. Proposed roles for lipid signals in mitochondrial fission and fusion

Phosphatidic acid (PA) produced at the mitochondrial surface through the cleavage of cardiolipin (CL) by MitoPLD facilitates Mitofusin (Mfn)-mediated mitochondrial fusion of the outer mitochondrial membrane. Fusion of the inner membrane is coordinately performed by l- and s-Mgm1 (Opa1), which requires CL for both localization and activity. The promotion of fusion by PA is terminated by its catabolism by Lipin 1b, which is recruited to the mitochondrial surface by a PA-binding domain and dephosphorylates PA to generate diacylglycerol (DAG), or by PA-Phospholipase A₁ (PA-PLA₁), which cleaves PA to generate LysoPA (LPA). Production of DAG or LPA at the expense of PA promotes mitochondrial fission; reduced Lipin 1b or PA-PLA₁ activity decreases fission, resulting in mitochondrial tubule elongation. Phosphatidylethanolamine (PE) is important for lipid mixing in mitochondrial membranes during fusion, although how this affects fusion efficiency is unknown. IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane. See text for additional details.