Roles for the lipid-signaling enzyme MitoPLD in mitochondrial dynamics, piRNA biogenesis, and spermatogenesis

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Phospholipase D (PLD), a superfamily of signaling enzymes that most commonly generate the lipid second messenger Phosphatidic Acid (PA), is found in diverse organisms from bacteria to man and functions in multiple cellular pathways. A fascinating member of the family, MitoPLD, is anchored to the mitochondrial surface and has two reported roles. In the first role, MitoPLD-generated PA regulates mitochondrial shape through facilitating mitochondrial fusion. In the second role, MitoPLD performs a critical function in a pathway that creates a specialized form of RNAi required by developing spermatocytes to suppress transposon mobilization during meiosis. This spermatocyte-specific RNAi, known as piRNA, is generated in the nuage, an electron-dense accumulation of RNA templates and processing proteins that localize adjacent to mitochondria in a structure also called intermitochondrial cement. In this review, we summarize recent findings on these roles for MitoPLD functions, highlighting directions that need to be pursued to define the underlying mechanisms. (BMB reports 2012; 45(1): 7-13)

INTRODUCTION
Long known as the powerhouse of the cell, mitochondria are now appreciated to regulate many different cellular functions including apoptosis, intracellular calcium signaling, and lipid synthesis and transport (1, 2). Mitochondrial shape, size and number are regulated by the balance of mitochondrial fusion and fission and affect mitochondrial function, which is important in mammalian health and disease (3, 4). MitoPLD, a member of mammalian phospholipase D (PLD) superfamily, was initially reported to facilitate mitochondrial fusion by hydrolyzing cardiolipin (CL) to generate phosphatidic acid (PA), constituting a novel lipid signaling pathway on the mitochondrial surface (5, 6). More recent work, however, has identified an additional function for MitoPLD in spermatogenesis (7-9).

Zucchini (Zuc), the Drosophila homolog of MitoPLD, was identified through screens for female infertility, which was subsequently determined to ensue from a critical role for Zuc in the PIWI-interacting RNA (piRNA) biogenesis pathway (10). The main function of piRNAs is to defend genomic integrity in germ-line cells by silencing transposable elements (11). Male mice lacking MitoPLD also have decreased piRNA biogenesis and meiotic arrest during spermatogenesis, analogous to the function of Zuc in Drosophila (8, 9). Nuage, an electron dense structure, is the subcellular organelle in which piRNA biogenesis takes place (12). Nuage is normally physically adjacent to mitochondria in spermatocytes; however, this relationship is disrupted in mice lacking MitoPLD (8, 9). Mutations in many of the components found in nuage impair piRNA biogenesis and derepress transposable elements (11, 13-20).

Intriguing questions are raised by the findings reported for MitoPLD and Zuc, centered on the control of mitochondrial shape and trafficking and why lipid-modifying enzymes on the surface of the mitochondria are required for piRNA biogenesis. We review here the current literature and experimental directions immediately apparent.

MAMMALIAN MitoPLD

Molecular and biochemical characterization
Phospholipase D superfamily members are found in prokaryotes, eukaryotes, and some viruses (reviewed in 21). The canonical PLD enzymatic activity is to hydrolyze the lipid phosphatidylcholine to yield choline and the signaling lipid PA, but some family members can hydrolyze other phospholipids or perform lipid synthetic or other actions involving hydrolysis or transfer at the phosphodiester bond linking the phosphate group to the headgroup. PA, a lipid second messenger, plays multiple roles in cellular functions including promoting cytoplasmic membrane fusion, and acting as a lipid anchor to recruit proteins to membrane surfaces and in some cases alter their functional activity (22). PA can also be converted through dephosphorylation by PA phosphohydrolases to diacylglycerol (DAG), another important signaling lipid in many cellular processes (23, 24). The classical isoforms of mammalian PLD, PLD1 and PLD2, were reported in the 1990’s (25, 26). Blast searches of the com-
pleted human genome, however, later revealed additional genes encoding the PLD catalytic domain, HIXK(X)₄D (HKD). One of these genes, MitoPLD (later also denoted pld6), was found to be located on the mitochondrial surface (5). However, in contrast to classical mammalian PLD family members which encode two half-catalytic HKD domains (26) and fold together to form the functional enzymatic unit (27), MitoPLD has only a single HKD half-catalytic site, requiring it to dimerize to create an active enzymatic complex (5). Interestingly, having only one copy of the HKD domain makes MitoPLD quite distinct from the classical PLD genes, and in fact closer in similarity to a prokaryotic branch of the superfamily known as “Nuc” (28). The bacterial Nuc genes function as endonucleases, hydrolyzing the phosphodiester bond in DNA that is analogous to the phosphodiester bond in phospholipids. Nonetheless, no group to date has been able to demonstrate any type of nuclease activity for MitoPLD (5, 9). The next most similar branch of the PLD superfamily to MitoPLD is a prokaryotic group of enzymes that synthesize cardiolipin (CLS); however, CLS activity also could not be demonstrated for MitoPLD (5, 29). What became apparent, though, was that alterations in lipid content in mitochondria were observed when MitoPLD was overexpressed, leading to the identification of a capability for MitoPLD to hydrolyze CL to generate PA (5).

In contrast to classical PLDs that have lipid-binding domains such as PX, PH, or PIP₂-binding motifs, MitoPLD is noteworthy for an N-terminal sequence that both localizes the enzyme to mitochondria and functions as a transmembrane domain to anchor MitoPLD into the mitochondrial surface. MitoPLD overexpression triggers mitochondrial aggregation (discussed below); replacing the key catalytic Histidine residue, H156N, with Asparagine, prevents this mitochondrial aggregation, indicating that it is MitoPLD’s enzymatic activity that mediates the change in morphology observed with overexpression.

**MitoPLD and mitochondrial dynamics**

Mitochondrial dynamics, including both fusion and fission, are very important in maintaining mitochondrial and cellular function, which are crucial for mammalian development and health (1, 30). Mitochondrial shape, size and number are determined by the rate and balance of mitochondrial fusion and fission. Increased fusion or decreased fission lead to elongated mitochondria, while decreased fusion or increased fission result in mitochondrial fragmentation. These morphological changes are linked to pathological states including cell apoptosis and neurodegenerative disease.

Mitofusin/fuzzy onion (MFN, fzo) was the first gene discovered to mediate mitochondrial outer membrane fusion (31). The mammalian MFN homologs, MFN1 and MFN2, are transmembrane GTPases located in the mitochondrial outer membrane. Mutations in MFN2 have been linked to the inherited peripheral neuropathy Charcot Marie Tooth disease (30). The C-terminal tail of MFN functions to tether opposing mitochondria as they approach each other during a fusion event, forming a dimeric, antiparallel coiled-coil structure (32). Electron microscopy revealed that the mitochondrial tethering effect creates an interface in which the adjacent mitochondria are separated by 16 nm (32). The GTPase is thought to mediate the fusion event subsequent to tethering.

Overexpression of MitoPLD also results in the juxtaposition of mitochondria. However, the mitochondria are separated by only 6 nm (5), and the ability of MitoPLD to create this mitochondria interface requires Mn proteins to be present. Thus, it was proposed that the MFN-driven tethering event leads to MitoPLD production of PA on the closely apposed mitochondrial outer membranes, triggering even closer apposition of the membranes to facilitate the MFN-GTPase fusion event. Supporting this hypothesis, overexpression of a catalytically-inactive (dominant-negative) MitoPLD mutant allele or use of MitoPLD RNAi led to mitochondrial fragmentation (5), and mouse embryo fibroblasts (MEFs) isolated from mice lacking MitoPLD exhibit shortened mitochondria (8). These findings indicate that MitoPLD facilitates mitochondrial fusion through a mechanism dependent on its enzymatic activity, although it is not absolutely required for fusion events to occur.

Support for a role for MitoPLD in fusion has recently been described in Drosophila (33). Mitochondrial fission plays a critical role in cellular apoptosis upstream of cytochrome c release. Dynamin-related protein 1 (Drp1) is the major mitochondrial fission mediator and is required for apoptosis (reviewed in 34). This pathway is relevant during dorsal closure, a complex morphogenetic movement and essential stage in Drosophila embryogenesis: apoptosis in cells in the amnioserosa triggers delamination of the cells which is the driving force for the dorsal closure (35). As reported by Muliyil and colleagues, increasing Drp1 expression or decreasing MitoPLD expression which promotes mitochondrial fragmentation, led to increased delamination, whereas decreasing Drp1 decreased delamination (33). This study showed that MitoPLD, by opposing mitochondrial fragmentation, has a functional role in the regulation of apoptosis and morphogenetic movements during embryogenesis in Drosophila.

Finally, PA is a dynamic lipid capable of being converted into other bioactive signaling lipids, one of which is diacylglycerol (DAG) (23). The family of cytoplasmic enzymes that mediate this activity is known as Lipin (23). Mutations in Lipin 1 cause fatty liver dystrophy and peripheral neuropathy in mice (36, 37). MitoPLD-generated PA recruits Lipin 1b to the mitochondria; Lipin 1b then converts the PA to DAG on the mitochondrial surface, terminating the fusion-promoting function of PA and shifting mitochondrial morphology towards increased fragmentation (8). Many mitochondrial fusion events are followed by a fusion event (38); the MitoPLD-PA-lipin1-DAG signaling cascade may play a role in the temporal-spatial changes of mitochondrial dynamics, in particular in mitochondrial dynamic-sensitive tissues such as fat, muscle and neurons. Further studies are needed to show how PA production regulates mitochondrial fusion, such as through recruiting proteins that have PA binding domains, or via the neg-