Mitochondrial membrane biogenesis: phospholipids and proteins go hand in hand

Vishal M. Gohil1,2,3 and Miriam L. Greenberg4

1Massachusetts General Hospital Center for Human Genetic Research, Boston, MA 02114
2Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA 02142
3Department of Systems Biology, Harvard Medical School, Boston, MA 02215
4Department of Biological Sciences, Wayne State University, Detroit, MI 48202


The mitochondrial inner membrane has a unique composition of proteins and phospholipids, whose interdependence is crucial for mitochondrial function. It is highly enriched in proteins specific to this membrane, the majority of which are encoded by the nuclear genome and imported from the cytosol. The lipid component contains the major classes of phospholipids found in all cell membranes, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylethanolamine (PS), and phosphatidylycerine (PG), as well as phosphatidylglycerol (PG) and cardiolipin (CL), which are located predominantly if not exclusively in the mitochondria (Zinser et al., 1991). PE, PG, and CL are synthesized “in house,” whereas the others must be imported. Mitochondrial membrane biogenesis, therefore, requires the coordinated import and synthesis of proteins as well as phospholipids. Historically, mitochondrial protein and phospholipid import and biosynthesis have been studied separately. In the last decade, however, it has become apparent that mitochondrial phospholipids play a fundamental role in the function and import of mitochondrial proteins, which in turn regulate the synthesis of these phospholipids.

CL, the signature phospholipid of the mitochondrion, is a dimeric phospholipid with the capacity to form nonbilayer structures in the presence of calcium ions. The CL biosynthetic pathway is well-characterized in Saccharomyces cerevisiae (see Fig. 1 A), and the availability of null mutants of the genes encoding the CL biosynthetic enzymes has made in vivo studies of CL function possible. From yeast mutant studies, it is now clear that CL has diverse mitochondrial functions in respiratory chain supercomplex stabilization, mitochondrial protein import, ceramide synthesis, aging and apoptosis, and the translation of electron transport chain components (Joshi et al., 2009). Moreover, CL also affects cellular processes not previously associated with mitochondrial function, including cell wall and vacuolar biogenesis (Zhong et al., 2005; Chen et al., 2008). The significance of CL in human health is apparent from clinical studies showing that perturbation of CL metabolism leads to the life-threatening disorder known as Barth syndrome (Schlame and Ren, 2006).

Although CL plays a key role in many mitochondrial functions, recent evidence suggests that at least some functions overlap with those of the phospholipid PE, which comprises >25% of total mitochondrial phospholipids. Mitochondrial PE is synthesized by the Psd1p-catalyzed decarboxylation of PS. Overlapping functions of CL and PE were suggested by the finding that deletion of PSD1 is synthetically lethal in CL-deficient yeast cells (Gohil et al., 2005), and may be attributed to the shared biophysical properties of PE and CL, both of which have the ability to form nonbilayer structures in membranes.

Correspondence to Miriam L. Greenberg: mgreenberg@wayne.edu

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Figure 1. Identification of novel regulators of mitochondrial nonbilayer-forming phospholipids. [A] Interdependence of CL biosynthesis and function. Four enzymatic steps catalyze CL biosynthesis in the inner mitochondrial membrane (IMM). The genes encoding three steps have been identified. Import machinery for proteins with N-terminal cleavable sequences (Tim23) or internal signals (Tim22) is also localized in the IMM. The newly identified Tam41 regulates Tim23- and Tim22-dependent protein import as well as CL biosynthesis, possibly by activating Cds1. CL, in turn, regulates protein import by maintaining the IMM potential by a mechanism not currently understood. CL stabilizes supercomplex assembly of complex III and IV, and stimulates complex IV activity. The pH gradient generated by respiratory chain activity increases CL synthesis by activating Crd1 synthase activity. [B] Genetic interactome of prohibitins regulates PE and/or CL levels. Genes identified in a synthetic genetic screen as genetic interactors of prohibitins (Phb1/2) consist of three classes that regulate (1) CL levels, (2) PE levels, or (3) both CL and PE. CDP-DG, cytidine diphosphodiacylglycerol; CL, cardiolipin; PA, phosphatidic acid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGP, phosphatidylglycerolphosphate; PGP, phosphatidylglycerolphosphate.
The functional importance of CL biosynthesis is underscored by the intricate regulation of CL synthesis via multiple novel mechanisms (Li et al., 2007). Evidence for cross-pathway control is seen in the down-regulation of CL synthesis by the phospholipid precursor inositol, possibly by posttranslational modification of PGS1 (He and Greenberg, 2004). Additionally, factors affecting mitochondrial biogenesis, such as carbon source and the presence of mitochondrial DNA, regulate the expression of PGS1 and CRD1 (Jiang et al., 1999; Zhong and Greenberg, 2003). A novel mechanism of regulation was demonstrated by the finding that the last step in CL biosynthesis, catalyzed by CL synthase, is stimulated by the increase in matrix pH that accompanies respiration (Gohil et al., 2004). This elegant mechanism clearly demonstrates the interdependence of CL biosynthesis and electron transport chain function; CL promotes supercomplex formation and cytochrome oxidase activity, which, in turn, up-regulates CL synthesis (Fig. 1 A). This theme is repeated in the work of Kutik et al. (2008), albeit in a different context.

The work of Kutik et al. (2008) identifies a new function for Tam41 (translocator and maintenance protein 41) in the regulation of CL biosynthesis. Tam41 is a component of the mitochondrial protein translocator system, peripherally attached to the inner mitochondrial membrane from the matrix side (Gallas et al., 2006; Tamura et al., 2006). Although previous studies have identified the role of Tam41 in the import of cleavable precursor-containing proteins via assembly and maintenance of the Tim 23 complex, its role in the import of noncleavable carrier proteins via Tim22 remained controversial (Gallas et al., 2006; Tamura et al., 2006). In seeking to resolve this controversy, Kutik et al. (2008) discovered that the tam41∆ mutant exhibited phenotypes that were remarkably similar to those of the CL-deficient crd1Δ mutant. As seen with the loss of CL, which is required for the optimal activity and assembly of the ATP-ADP carrier (AAC; Claypool et al., 2008), the tam41Δ mutant exhibited a marked absence of AAC oligomers, as visualized by blue native PAGE. Mitochondrial respiratory chain supercomplex assembly was also perturbed in the tam41Δ mutant. These shared phenotypes suggested a possible role of Tam41 in CL regulation. Indeed, phospholipid analysis of mitochondria isolated from tam41Δ cells showed an almost complete absence of CL and its precursor PG. The expression of Crd1 and Pgs1 proteins was unaffected by the loss of Tam41; however, the accumulation of PA in tam41Δ mitochondria suggested that regulation may occur at the level of CDP-DAG synthase (Fig. 1 A). These data suggest that Tam41 is primarily required for PG and CL biosynthesis, and that defects in protein import in tam41Δ are a secondary consequence of the loss of these lipids. However, it is likely that Tam41 has an additional function, as tam41Δ, unlike pgs1Δ, exhibits a true petite negative phenotype that cannot be rescued by 1 M sorbitol (Gallas et al., 2006). In light of earlier work showing that CL is required for maintenance of the mitochondrial membrane potential (ψ) and the import of proteins across the inner mitochondrial membrane (Jiang et al., 2000), the current report (Kutik et al., 2008) shows that the function of CL in mitochondrial protein import is interdependent with the regulation of CL biosynthesis by components of the mitochondrial protein import apparatus (Fig. 1 A). Tam41, therefore, has a dual role in maintaining mitochondrial structural integrity by regulating both protein and phospholipid composition. The unique regulatory function of this protein would allow the coordinated biosynthesis of mitochondrial components and prevent the formation of structurally and functionally aberrant mitochondria.

A fascinating new dimension of CL regulation is seen in the study of Osman et al. (see p. 583 of this issue), which shows that the biosynthesis of CL and PE is coordinately regulated and tied to the prohibitins (Phbs). Phbs are multimeric, ringlike structures present in many different cellular membranes, including the mitochondrial inner membrane. The rings, which consist of multiple copies of Phb1 and Phb2 subunits with a molecular mass of >1 MD, play a role in cell proliferation, cristae morphogenesis, and the functional integrity of mitochondria (Merkwirth and Langer, 2009). In *S. cerevisiae*, Phb complexes associate with mitochondrial proteolytic machinery, thereby controlling protein turnover (Steglich et al., 1999). They also bind to mitochondrially translated proteins, raising the possibility that they have a chaperone-like function (Nijtmans et al., 2000). The biochemical activity of Phbs remains unknown.

To gain insight into the function of Phbs, Osman et al. (2009) used the synthetic genetic array strategy to identify genetic interactions with Phb. In this manner, they identified 35 genes that are required for the survival of Phb-deficient cells. These genes encode four classes of proteins: (1) respiratory chain assembly proteins, (2) mitochondrial morphology regulating proteins, (3) uncharacterized proteins that the authors name genetic interactors of prohibitins (GEP), and (4) proteins with diverse functions, including enzymes that synthesize CL and PE. The observed synthetic lethality of Phb with genes encoding PE- and CL-synthesizing enzymes suggested that the other genes identified in the screen may play an important role in phospholipid regulation.

The first indication of linkage between Phb function and phospholipid regulation came from the finding that synthetic lethality of phb1Δgep1Δ cells could be rescued by increased expression of CHO1, which encodes PS synthase. This enzyme catalyzes the synthesis of PS, the precursor of PE. Consistent with this, the mitochondrial phospholipid profile of gep1Δ cells showed increased PE turnover and significantly reduced PE levels, which were restored by the overexpression of CHO1. A further link between Phb and phospholipid synthesis was apparent in the observation that loss of the GEP gene family member UPS1 resulted in a dramatic sevenfold reduction of CL without affecting PE levels. Interestingly, deletion of GEPI restored normal CL levels in ups1Δ mitochondria, which suggests coordinated regulation of mitochondrial nonbilayer-forming phospholipids by GEP family proteins. Mitochondrial phospholipid profiles in the remaining Phb-interacting genes identified three functional classes of genes that regulate levels of CL, PE, or both (Fig. 1 B). This study, therefore, highlights critical overlapping roles of Phbs and the phospholipids CL and PE in maintaining mitochondrial inner membrane integrity. These findings are completely consistent with earlier studies showing synthetic lethal interactions of Phb with the PE-synthesizing enzyme Psd1p (Birner et al., 2003), and between mitochondrial PE and CL biosynthetic pathways (Gohil et al., 2005). Based on these data, the authors proposed the presence of defined lipid clusters in the mitochondrial membrane facilitated by the ringlike Phbs. The demonstration of such structures and the function of these PE-CL-rich domains remains an open question.