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The topology and regulation of cardiolipin biosynthesis and remodeling in yeast

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ABSTRACT

The signature mitochondrial phospholipid cardiolipin plays an important role in mitochondrial function, and alterations in cardiolipin metabolism are associated with human disease. Topologically, cardiolipin biosynthesis and remodeling are complex. Precursor phospholipids must be transported from the ER, across the mitochondrial outer membrane to the matrix-facing leaflet of the inner membrane, where cardiolipin biosynthesis commences. Post-synthesis, cardiolipin undergoes acyl chain remodeling, requiring additional trafficking steps, before it achieves its final distribution within both mitochondrial membranes. This process is regulated at several points *via* multiple independent mechanisms. Here, we review the regulation and topology of cardiolipin biosynthesis and remodeling in the yeast *Saccharomyces cerevisiae*. Although cardiolipin metabolism is more complicated in mammals, yeast have been an invaluable model for dissecting the steps required for this process.

1. Introduction

The unique phospholipid cardiolipin (CL) is required for the efficiency of a number of mitochondrial processes (Claypool and Koehler, 2012). CL is unique for a number of reasons: (1) unlike most other phospholipids which are synthesized in one or a few cellular locations, then disseminated throughout a cell's membranes, CL by and large remains in the mitochondrion, its site of synthesis; (2) CL is essentially a lipid dimer; it consists of two phosphate headgroups, which are attached by a glycerol moiety, and four acyl chains; and (3) after its synthesis, CL undergoes acyl chain remodeling, where acyl chains are removed by a lipase and replaced by a transacylase or acyltransferase, resulting in the establishment of only a few molecular forms of CL in a cell or tissue. Surprisingly, the acyl chain specificity of the lipase has never been demonstrated (Beranek et al., 2009), and the transacylase tafazzin has no acyl chain specificity (Schlame, 2012), although tafazzin from *Drosophila* has been shown to preferentially catalyze transacylation reactions on curved membranes leading to the establishment of CL with unsaturated acyl chains, which were proposed to decrease lipid disorder in areas of high curvature (Schlame et al., 2012a). Curiously, the final molecular form of CL varies between organisms and even between cell types within the same organism.

CL serves the cell in multiple capacities: it associates with all the major proteins of the mitochondrial respiratory chain and thereby increases the efficiency of electron flow and ADP/ATP exchange (Acehan et al., 2011; Bazan et al., 2013; Claypool et al., 2008b; Fry and Green, 1981; Jiang et al., 2000; Schagger et al., 1990; Schwall et al., 2012; Yu and Yu, 1980), modulates the catalytic activities and stability of interacting proteins (Claypool et al., 2008b; Gomez and Robinson, 1999; Jiang et al., 2000; Pfeiffer et al., 2003; Wenz et al., 2009), is critical for the biogenesis of mitochondrial proteins (Gebert et al., 2009; Jiang et al., 2000; Joshi et al., 2009), facilitates mitochondrial fission/fusion (Ban et al., 2010; DeVay et al., 2009; Joshi et al., 2012), and is involved in the maintenance and plenitude of cristae morphology (Acehan et al., 2007, 2009; Mileykovskaya and Dowhan, 2009).

In addition to the importance of CL in promoting and maintaining normal mitochondrial function, alterations in CL metabolism have been associated with ischemia and reperfusion, heart failure, diabetic cardiomyopathy, and Barth syndrome (Chicco and Sparagna, 2007; Claypool et al., 2006; Gu et al., 2004; Paradies et al., 1997; Schlame and Ren, 2006). Barth syndrome is caused by mutations in tafazzin (*TAZ1*), and patients present with cardio- and skeletal myopathy, neutropenia, 3-methylglutaconic aciduria, and abnormal mitochondria (Barth et al., 1983; Schlame and Ren, 2006).

Abbreviations: CCCP, carbonyl cyanide 3-chlorophenylhydrazone; CDP-DAG, CDP-diacylglycerol; CL, cardiolipin; ERMES, ER–mitochondriaencounter structure; IM, inner membrane; IMS, intermembrane space; OM, outer membrane; PA, phosphatidic acid; PG, phosphatidylglycerol; PGP, phosphatidylglycerolphosphate; UAS_{INO}, inositol sensitive upstream activating sequence.

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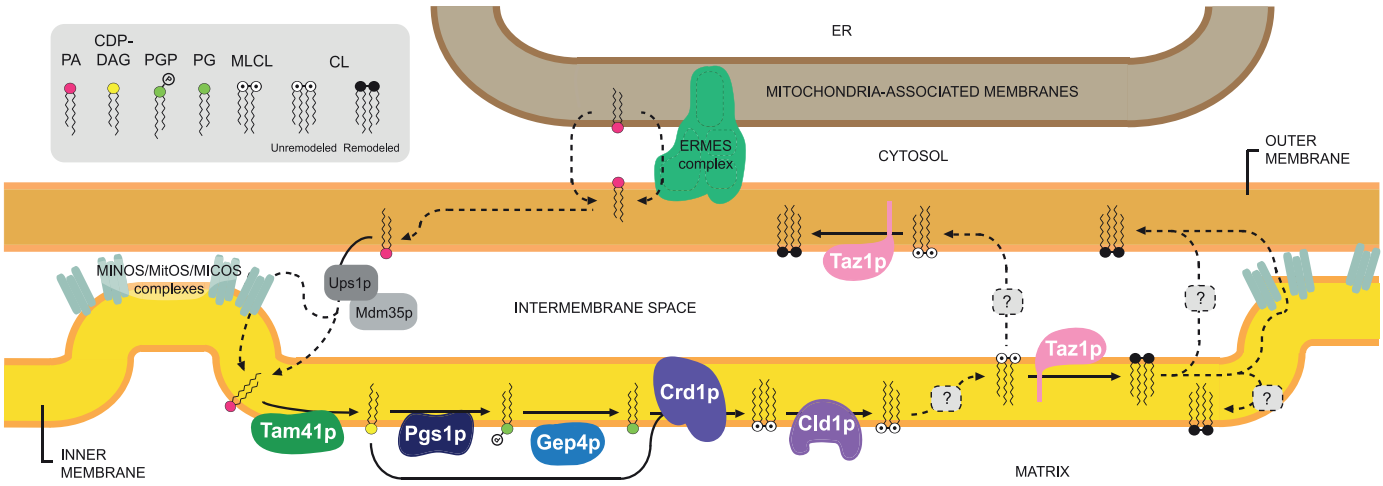

Fig. 1. The topology of CL biosynthesis and remodeling. Phosphatidic acid (PA) is synthesized in the ER and translocates to mitochondria in a process that is influenced by the ERMES (ER–mitochondria encounter structure) complex. Ups1p/Mdm35p heterodimers transport PA from the OM to the IM, potentially at contact sites (established by MINOS/MICOS/MitOS complexes). PA is converted to CDP-diacylglycerol (CDP-DAG) by Tam41p on the matrix-facing leaflet of the IM. CDP-DAG is used to generate phosphatidylglycerolphosphate (PGP) by Pgs1p. PGP is dephosphorylated to phosphatidylglycerol (PG) by Gep4p. PG and another CDP-DAG are condensed to form unremodeled CL by Crd1p. CL is deacylated by Cld1p on the matrix-facing leaflet of the IM, forming MLCL. *Via* an unknown mechanism, MLCL must flip to the IMS-facing leaflet of the IM or be transported to the OM to gain access to the transacylase Taz1p, which regenerates CL. Multiple rounds of deacylation/reacylation result in remodeled CL which is enriched in unsaturated acyl chains. CL achieves its final distribution on both leaflets of the IM and OM through currently ill-defined mechanisms. The depicted topology of Pgs1p has not been experimentally verified. Solid lines indicate known pathways. Dashed lines delineate potential but currently unknown phospholipid transport processes.

Much of the knowledge of CL biosynthesis and remodeling comes from studies in yeast. In addition to the "usual" advantages of using yeast as a model system (Baile and Claypool, 2013; Botstein and Fink, 2011), yeast are viable in the absence of CL and CL precursor phospholipids (Chang et al., 1998a,b; Jiang et al., 1997; Osman et al., 2010; Tuller et al., 1998) whereas in higher eukaryotes CL is required for life (Zhang et al., 2011). Although CL biosynthesis and remodeling are highly conserved between yeast and higher eukaryotes, there are still a few differences. There are no orthologs of Gep4p, the phosphatidylglycerolphosphate (PGP) phosphatase, or Cld1p, a CL lipase, in higher eukaryotes (Beranek et al., 2009; Osman et al., 2010). However, the phylogenetically unrelated PTPMT1 performs the same function as Gep4p (Zhang et al., 2011); and a calcium-independent phospholipase A_2 has been implicated as a CL lipase (Malhotra et al., 2009; Mancuso et al., 2007; Schlame et al., 2012b), although its exact role in CL remodeling remains nebulous (Kiebish et al., 2013). Additionally, only the tafazzin-mediated CL remodeling pathway exists in yeast, while additional remodeling enzymes have been identified in mammals (reviewed in Claypool and Koehler, 2012). Thus, while yeast have been useful in dissecting this process, the complexity and multitude of players in mammalian CL remodeling suggest that there is still much to discover.

With the recent characterizations of Cld1p, Gep4p and Tam41p (Beranek et al., 2009; Osman et al., 2010; Tamura et al., 2013), it is likely that all of the proteins catalyzing CL synthetic or remodeling reactions have been identified in yeast; however, many questions regarding the regulation of this process, as well as the topology and trafficking of CL and its precursors, remain (Fig. 1).

2. Delivering precursor phospholipids to the IM

CL biosynthesis requires CDP-diacylglycerol (CDP-DAG), which is formed from phosphatidic acid (PA) and CTP by a CDP-DAG synthase (Shen et al., 1996). Yeast contain two CDP-DAG synthases: Cds1p in the ER (Kuchler et al., 1986), and the recently characterized Tam41p in the mitochondrial inner membrane (IM; Tamura et al., 2013).

Although CDP-DAG (containing an NBD moiety) is able to be translocated from the ER to the IM *in vitro*, this process is inefficient (Tamura et al., 2013). The very low abundance of CL in Δ*tam41* yeast (Kutik et al., 2008; Tamura et al., 2012) suggests that if Cds1p-derived CDP-DAG contributes to CL biosynthesis, its role is very minor. Tam41p is peripherally associated with the matrix side of the IM (Table 1) (Gallas et al., 2006; Tamura et al., 2013). Thus, Tam41p activity requires that its substrate, PA, be transported from the ER to the matrix-facing leaflet of the IM. Phospholipid transport between the ER and mitochondrial outer membrane (OM) was suggested to be mediated by the ER–mitochondria encounter structure (ERMES) complex which physically tethers the two organelles (Kornmann et al., 2009). Indeed, loss of any ERMES complex subunit (Mdm10p, Mdm34, Mdm12p, or Mmm1p) alters the mitochondrial phospholipid profile, including reducing CL (Kornmann et al., 2009; Stroud et al., 2011; Tamura et al., 2012). However, its direct role in phospholipid transport has recently been challenged (Nguyen et al., 2012; Voss et al., 2012). Further, defects caused by the loss of a functional ERMES complex can be rescued by expressing an artificial ER–mitochondria tether, suggesting that the ERMES complex facilitates phospholipid transport by forming close contact sites between the two membranes, rather than directly transporting phospholipids (Kornmann et al., 2009; Nguyen et al., 2012; Voss et al., 2012). Notably, these studies focused on the transport of phosphatidylserine from the ER to mitochondria (and phosphatidylethanolamine to the ER after is decarboxylation in mitochondria). Thus, the mechanisms of PA and CDP-DAG transport from the ER to mitochondria, and the players involved, including a direct assessment of the role of the ERMES complex, remain to be discovered.

To reach to IM, CL precursor phospholipids must traverse the OM, but little is known about this process. Phospholipid exchange between leaflets of purified OM vesicles is rapid, suggesting that proteins mediate this process. However, treatment with proteases or with sulfhydryl reactive compounds does not inhibit transbilayer movement across the OM (Janssen et al., 1999).

PA is transported from the OM to the IM by the intermembrane space (IMS) resident protein, Ups1p (Connerth et al., 2012). Mdm35p binds Ups1p, facilitating its import into the IMS and preventing its proteolytic degradation (Potting et al., 2010; Tamura et al., 2010). Although Ups1p/Mdm35p dimers can bind negatively charged phospholipids, only PA is transported *in vitro*,